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Official Organ of
The American Phytopathological Society

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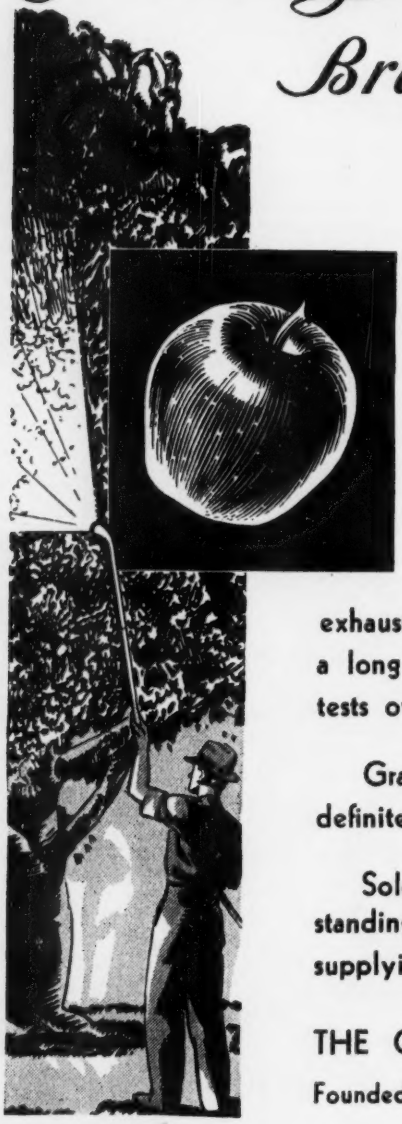

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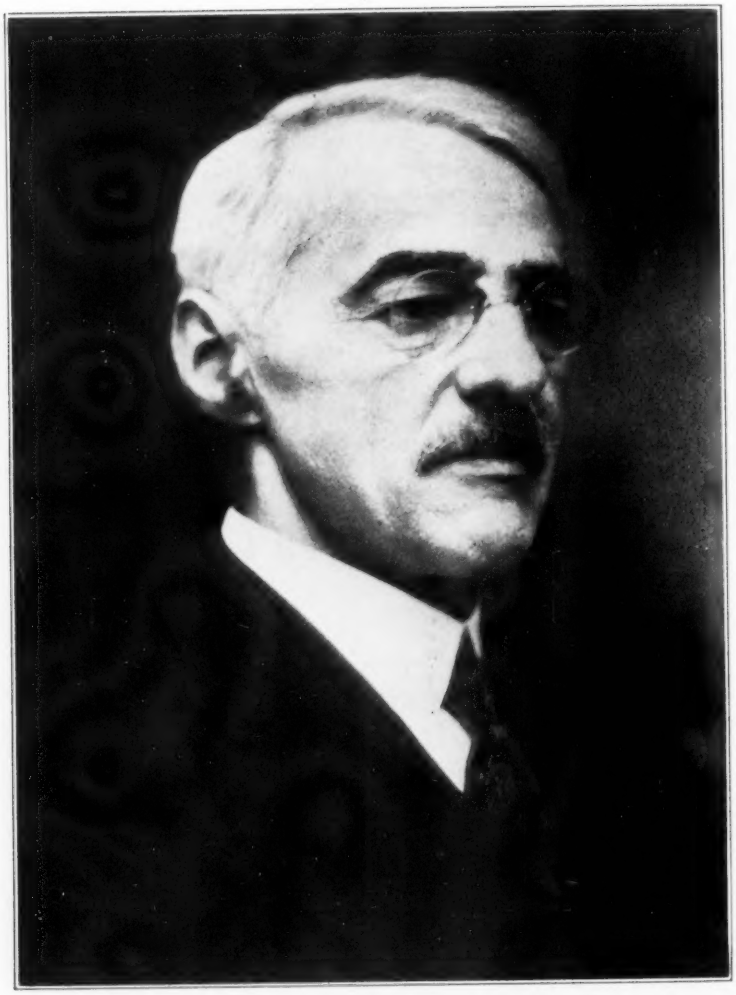

CALVIN HENRY KAUFFMAN
1869–1931

PHYTOPATHOLOGY

VOLUME 22 APRIL, 1932 NUMBER 4

CALVIN HENRY KAUFFMAN
1869–1931

E. B. MAINS

Calvin Henry Kauffman was born March 1, 1869, on a farm in the little community of Kimmerling's near Lebanon, Pennsylvania. He died June 14, 1931, at the age of 62, at his home in Ann Arbor, Michigan, following an illness of 16 months' duration. He came from a long line of sturdy Pennsylvania German ancestors who settled in this country in the early days.

Doctor Kauffman received his early schooling in the little country school adjacent to the Kimmerling's church. As a young man he took an active part in the social life of the community. His musical talents found expression in playing the church organ and in directing singing schools in the neighborhood. At the age of 18 he began his career as a teacher and, for 3 years (1887–1890), taught in the secondary schools of Lebanon.

In 1890 he entered the Palatinate College, Myerstown, Pennsylvania, where he spent 2 years preparing for entrance to Harvard University. In 1892 he matriculated at Harvard and the 4 succeeding years were spent there. He specialized in languages and was graduated with distinction in 1895 with an A.B. degree. In 1895 he married Elizabeth Catharine Wolf, by whom he is survived.

Following his graduation he held the position of principal in a secondary school in Lebanon for 2 years (1896–1898). He then taught for 2 years (1898–1900) in a high school at Decatur, Indiana, and the following year in a normal school at Bushnell, Illinois.

Although he devoted but little of his time to science while in the University, he very early showed a special interest in botany. Before entering Harvard he, independently, made a study of the flora about Lebanon and started his herbarium of flowering plants. It was at Bushnell that his interest was turned definitely into the field of science. This was instigated partly by teaching a course in chemistry. He delighted in telling of a successful laboratory that he constructed from an old kitchen. While at Bushnell he acquired a copy of Atkinson's "Mushrooms" and spent considerable time studying the agarics of the vicinity.

As a result he decided to train himself in science and the following year (1901–1902) he spent at the University of Wisconsin where he came under

the influence of Professor R. A. Harper, who first aroused his interest in the Phycomycetes. The next year he went to Cornell University as a personal assistant to Professor G. F. Atkinson. Here he had as fellow students H. H. Whetzel, H. S. Jackson, J. M. Van Hook, and Charles Thom. For 2 years (1902–1904) he studied with Professor Atkinson and became keenly interested in the study of agarics.

In 1904 he accepted an instructorship in the botany department of the University of Michigan. He threw himself enthusiastically into his mycological studies and continued his field collecting as he had been doing so thoroughly at Cornell. He renewed his studies of a problem concerning the physiology of Saprolegnia species, which he completed and presented for his doctorate thesis in 1907. The dissertation was entitled "A Contribution to the Physiology of the Saprolegniaceae with Special Reference to the Variations of the Sexual Organs." Through the use of proper nutrient media he was able to control with remarkable accuracy the development of the species studied and change them from vegetative to reproductive phases, at will. In this investigation he became acquainted with the work of George Klebs, with whose theories his results agreed. Throughout the rest of his life the Klebsian principles dominated his own physiological studies and those of his students.

While at Cornell he became interested in the difficult genus Cortinarius, and in 1905 he published his first paper on the agarics, entitled "The Genus Cortinarius, a Preliminary Study." During these studies his attention was attracted to a mycorrhiza caused by *Cortinarius rubripes* Kauff., and he published his observations in a paper entitled "Cortinarius as a Mycorrhiza-Producing Fungus." The interest in mycorrhiza thus aroused was always maintained and was communicated to many of his students in forest pathology. For several years he devoted his attention to taxonomic studies of fungi of Michigan with special reference to the Agaricaceae. His field work took him to many parts of the State. Studies based upon these collections were published in a series of papers that appeared from year to year in the Michigan Academy Reports under the title "Unreported Michigan Fungi." His studies in agarics resulted in the publication in 1918 of a work of 2 volumes, "The Agaricaceae of Michigan," which contains critical descriptions of 884 species and is illustrated with 172 plates. This work may be classed as one of the major contributions to the knowledge of the Agaricaceae in this country.

The summer of 1908 he spent in Europe acquainting himself with the agarics of northern Europe.

In 1914 he spent a very profitable summer collecting and studying fungi in Peck's favorite collecting grounds at North Elba, New York. After

1914 he turned his attention more and more to the study of the flora of the Rocky Mountain region and the Pacific Coast States. Accompanied by his students, he conducted expeditions in Washington (1915), Tennessee and Kentucky (1916), Colorado (1917, 1920, 1928), Idaho, Wyoming, and Oregon (1922), Wyoming (1923), North Carolina and Tennessee (1924), and Upper Peninsula of Michigan (1927, 1929). These expeditions furnished him with an abundance of material critically studied in the field upon which his monographic studies and fungus floras were based. He described more than 200 new species of fungi. On these expeditions he collected not only fungi but also mosses, ferns, and flowering plants.

While his main interest was chiefly in the field of mycology, he contributed a number of papers on pathological subjects. The number of these, however, is by no means the measure of his interest in this field of research. He developed an outstanding course in forest pathology and stimulated his forestry students to keep alert to new problems in forest pathology. His interest in pathology is reflected in the problems of several of his graduate students who have published the results of investigations in this field. From 1917–1919 he was on leave from the University of Michigan, serving as pathological inspector on the Federal Horticultural Board.

In 1912 he was advanced to the rank of Assistant Professor and in 1920 to Associate Professor. In 1923 he was promoted to the rank of Professor of Botany.

In 1921 he was appointed Director of the University Herbarium, which, under his leadership, made rapid progress. The organization of the Herbarium as a strong research unit was one of his major achievements. His keen interest in the herbarium is indicated by the fact that he deposited there his personal herbarium, which consisted of thousands of specimens.

Throughout his life Doctor Kauffman retained his early interest in teaching. At Michigan he developed courses in algae, mosses, liverworts and ferns, and in mycology and forest pathology. His broad botanical interests are reflected in the diversity of the problems of his doctorate students. He not only had the ability to transmit his knowledge to his students but he inspired them to seek for information and to think for themselves. He always took an active interest in their problems and stimulated and encouraged them by his criticisms. His devotion to research, his boundless enthusiasm, and his unusual ability to detect the significant facts in research will be remembered as outstanding characteristics by all who came in contact with him.

Fortunately, a few weeks before his illness, his doctorate students expressed to him a little of their affection for him. At that time they pre-

sented a bas-relief of Doctor Kauffman to the University as a token of their appreciation of him as friend and teacher.

He was a member of The American Phytopathological Society, a fellow of the American Association for the Advancement of Science, and a member of the American Botanical Society, Torrey Botanical Society, Société Linnéène de Lyon, Washington Botanical Society, Michigan Academy of Science, Arts and Letters, Sigma Xi, and the American Forestry Association.

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EFFECTS OF ELECTROMAGNETIC WAVES ON FUNGI[1]

FRANK H. JOHNSON

INTRODUCTION

In view of the increasing interest concerning the action of various rays on fungi the experiments described in this paper were undertaken with the idea of finding out to what portions of the electromagnetic spectrum three fungi, heretofore not studied from this point of view, were most susceptible to variations as a result of the rays. The species used were: *Collybia dryophila* Fr., *Sclerotium bataticola* Taub., and *Fusarium batatatis* Wr. Although previous investigators have, for the most part, studied in detail the effects of one particular type or sometimes two types of rays, the object of this study was, primarily, more to determine in a comprehensive way the reactions of the organisms to wave lengths representative of the major kinds of rays throughout as much of the electromagnetic spectrum as could be worked with in the laboratory, with certain limitations imposed by the difficulties of technique for working with some parts of the spectrum. The total range of wave lengths covered in this study extended from only a small fraction of an A.u. in the case of gamma rays to Hertzian waves of 50 and 100 m.

Inasmuch as a different technique was necessitated for experimentation with the different kinds of rays, only a few words concerning general methods need be mentioned here.

A sweet-potato-agar medium was used for pure cultures of the organisms. Except where stated otherwise, the pH of the medium was adjusted to 6.01 with a quinhydrone electrode. Inoculations, except in a few cases as described, were made by transferring a small bit of mycelium-bearing agar from a pure culture to the center of a Petri dish containing approximately 20 cc. of sterile medium. Irradiations were carried out as described under the separate headings.

REVIEW OF LITERATURE

a. *Gamma and X-rays.* The action of gamma and X-rays on fungi has not, apparently, been very extensively investigated, although considerable work has been done with *Bacterium tumefaciens* Smith & Towns. Actino-

[1] The writer wishes to acknowledge with much appreciation the assistance and generous cooperation, both in the use of apparatus and valuable suggestions for completing this work, rendered by the following: The staff of the Botany Department, N. C. State College of Agriculture and Engineering, particularly Dr. R. F. Poole and Dr. B. W. Wells; the Biology Department, Princeton University, especially Dr. Cary, Dr. Harvey, and Dr. Butler; the Physics Department, Princeton University, especially Professors Shenstone, Morris and Boice.

mycosis of the face and neck, caused by *Actinomyces bovis* Harz., has been successfully treated by a concentrated preparation of radium, filtered through 1 to 2 mm. of lead (10). Miescher found that prolonged exposure to radium rays retarded and finally suppressed development of cultures of *Trichophyton gypseum* and *Achorion gypseum* Bodin (14). Rivera (22) found that a capillary tube containing radium, suspended in test-tube cultures of *Bact. tumefaciens*, *Bact. fluorescens* (Flügge) Lehm. & Neum., and *Penicillium crustaceum* Fr., respectively, inhibited the growth of the organisms in the part directly exposed to the rays and concluded that gamma rays suppressed the growth and then beta rays killed the organisms.

Pichler and Wöber (18) tried the use of ultra-violet, X-rays, and radium for the control of bunt of wheat and smuts of barley, oats, and maize. Radium yielded only negative results, but successful treatment was obtained by ultra-violet and X-rays, especially in acid- and oxygen-containing solution, against *Tilletia tritici* (Bjerk.) Wint., *Ustilago nuda* (Jens.) Kell. & Sw., *U. hordei* (Pers.) Kell. & Sw., *U. avenae* (Pers.) Jens., and *U. zeae* (Beckm.) Ung. In X-ray experiments with *T. tritici* and *T. levis* Kühn, Rivera (21) obtained only negative results. Nadson and Philipov (15) found that sexual reproduction in pure cultures of young *Mucor genevensis* Lendn. and *Zygorrhynchus Moelleri* Vuill. was almost completely inhibited by 30 to 45 minutes of irradiation by X-rays, while weaker radiation stimulated development and induced mutations. The mutant characters were maintained in some cases through as many as 13 successive generations from spores of the mutants.

b. *Ultra-violet rays.* The portion of the spectrum usually thought of, and considered in these studies, as the ultra-violet region, extends from approximately λ 3900 to λ 1850.[2] The wave lengths below λ 1850, down to λ 1250, are generally designated Schumann rays. Ordinary atmosphere is practically opaque to wave lengths shorter than λ 1850 because of the high absorption of oxygen, and a special technique is necessary for studying the action of the waves on living material. Comparatively little is known concerning the physical properties of the waves between the very "soft," or long X-rays, which, above 10 A.u., have only a very short range in air, and the lower limits of the Schumann region into which the X-rays merge. The technique would be exceedingly difficult for studying the action of electromagnetic waves between 1 and 1250 A.u. on living organisms.

The first investigation of the effects of Schumann rays on protoplasm was made by Bovie (3), who studied the action of this light on a number of organisms, including rotifers, Spirogyra, amoeba, and spores of Penicillium, *Cephalothecium roseum* Cda., and *Monilia* sp. Spores were irradi-

[2] The Greek letter λ is used to denote wave length, here referring to Ångström units.

ated *in vacuo,* while the other organisms were placed in a thin film of water on a flourite window of a hydrogen-discharge tube. The rays were found to have a lethal action, the time required for killing varying both with the species and individual organism, though, in general, a small organism was more easily killed than a large one. Sphaerella-like swarm spores were killed almost instantly. Bovie did not succeed in killing the tan-colored spores of *P. brevicaule* Sacc. or the black spores of *Stemphylium* sp. and concluded that it was because the Schumann rays did not have sufficient penetrating power to pass through the colored cell walls. By using a rock-salt screen, cutting off waves below λ 1800, it was demonstrated that the shorter wave lengths were the more destructive. A remarkable effect of the Schumann rays was their cytolytic action on cells. Bovie distinguished three types of photocytolysis in ciliated infusoria, which were quickly cytolyzed. Spores of Monilia, after exposure to the rays, when dry, and then allowed to absorb water until turgid, took on a coarsely granular, coagulated appearance.

Except for the Schumann region, more work has been done concerning the effects of ultra-violet light on fungi than any other portion of the spectrum, and it is under the influence of these rays that the organisms are, apparently, most susceptible to variation. By and large, the action of this light may be considered as of two kinds, which, to a certain extent, may take place concomitantly: it may be destructive, involving a suppression of growth or lethal effects on the mycelium or spores, or it may be stimulating to the formation of fruiting bodies. Lethal effects have been reported by a number of investigators. Fulton and Coblenz (9), working with a number of species of fungi, showed that ultra-violet radiations were destructive to spores after a comparatively short exposure on the surface of hard nutrient agar in Petri dishes. They found that the lethal dose varied with the species but that an interval of 45 to 60 seconds was generally quite efficient. They concluded that the fungicidal effectiveness of ultra-violet light depends on the intensity of the radiation and the wave lengths of the component rays. Lethal action was found to begin around 365 μ (undoubtedly $\mu\ \mu$ was meant), as an upper limit, and to increase in effectiveness with decrease in wave length. Growing mycelium was found more sensitive than spores. Bovie (2) found that bacteria and spores of various fungi were killed by exposures of approximately 10 minutes to wave lengths shorter than λ 2925 but were not killed after 2-hour exposures to wave lengths above λ 3175. Tanner and Ryder (25) found yeast fungi quite susceptible to the lethal action of ultra-violet light, though, in general, less so than bacteria. The lethal dose varied with the species, ranging from 1.5 to 20 minutes. Pigmented yeasts were found to be more resistant than those without pigment.

Their results indicated a direct relation between the size of the cell and its resistance to the light. Chyurlia (5) reported the alleviation of a case of bronchomycosis caused by *Monilia Krusei* Castel. under ultra-violet treatment, and Chavarria and Clark (4) have noted slightly inhibitory effects of ultra-violet light on nonpigmented pathogenic fungi of the skin, although the visible and near ultra-violet were stimulating in moderate doses. Lethal effects have been recorded by Sibilia (23) on the spores of *Fusarium echinosporum* Sib. and *F. fuliginosporum* Sib. Dufrénoy (7) observed cytolysis of the zoospores of Blepharospora irradiated for 2 to 3 minutes in a thin film of water. Lethal effects on the spores of *Glomerella cingulata* (Stonem.) S. and v. S. with a collapse and death of aerial mycelium in colonies 2 to 7 or 8 days old, were found by Stevens (24). Petri (16) tested the resistance of conidia of oak mildew to ultra-violet light and found that, although they germinated normally after exposures to wave lengths between λ 3900 and λ 4000, they were destroyed effectively by wave lengths from λ 2300 to λ 2500, with less effective action between λ 3130 and λ 3660. Acid media accentuated the destructive action. There was a high resistance to direct solar light.

In experiments conducted on a number of different fungi, Weston and Halnan (26) found in each case that the growth of the cultures exposed to the range of ultra-violet light passing through Sanalux or Vitaglass Petri dish covers from a quartz mercury-vapor lamp was suppressed. They noted that the mycelium was not killed but rendered "dormant" by the rays, and there was a tendency to grow deep into the medium to avoid the light. Porter and Bockstahler (19) found an inhibition of growth, together with a "collapse and probable death" of aerial hyphae in cultures exposed to the full range of ultra-violet light from a quartz mercury-vapor lamp. They noted, however, that with the exception of *Sclerotinia* sp., which was not killed by an irradiation of 30 minutes, their fungi were not actually killed after exposures of 60 minutes.

In addition to lethal or inhibitory action of ultra-violet light on the growth of fungi, the action on reproductive bodies is significant. Stimulation of the production of acervuli and perithecia in *Glomerella cingulata,* when exposed to the full range of ultra-violet light for as little as 2.5 to 4 seconds, has been reported by Stevens (24). The production of pycnidia in Coniothyrium was brought about earlier than normal by irradiations as short as 10 seconds. The effects of ultra-violet light on sporulation in *Fusarium cepae* Hanz. and *Macrosporium tomato* Cke. have been pretty thoroughly worked out by Ramsey and Bailey (20), who found that the most efficient wave lengths for increasing sporulation lay between λ 2535 and λ 2800. But they also found that abundant sporulation could be in-

duced by wave lengths above λ 3120 by exposure to direct sunlight and conclude that, if the intensity is great enough, wave lengths shorter than λ 3120 are not necessary for stimulation of spore production.

There does not seem to be any evidence that any of the variations quoted above have been due to a change in the culture medium as a result of the irradiations. There is evidence, however, that ultra-violet radiations produce changes in certain media that may affect the growth of the organism. Euler (8) obtained a better growth of *Penicillium glaucum* Lk. and *Rhizopus chinensis* Saito on media that had been irradiated for 2 minutes but a decreased growth on media that had been irradiated for 2 hours. Petri (17) found that the growth of *Blepharospora cambivora* Petri on carrot agar, sterilized and irradiated, was more vigorous. The same was true of *Deuterophoma tracheifila* Petri on boiled, irradiated milk.

c. *Visible light, infra-red, and Hertzian waves.* While there has been comparatively little investigation of the action of visible light and infra-red rays on fungi, it does not appear that such organisms are particularly sensitive to radiations in this region of the spectrum. Variations in the shape of colonies of *Microsporon audonini* Gruby and *Achorion schoenleinii* Leb. were found by Berde (1) to take place under various types of illumination. Porter and Boekstahler (19) found little marked differences in colonies of several fungi exposed continuously to various wave lengths in the visible spectrum, and to infra-red, though there was a tendency for cultures to grow less rapidly under any of the lights used than in darkness, especially so in cultures exposed to blue-green light. The most sensitive part of the colony to inhibition was the aerial hyphae. No change was observed in sporulation of *Colletotrichum lindemuthianum* (Sacc. & Magn.) Br. and Cav. under any of the lights, but a range of sensitivity to various wave lengths was noted in *Cephalothecium roseum* Cda. Cultures exposed through filters transmitting only infra-red rays underwent no modification. Cooper and Porter (6) found the vegetative growth of 2-day old cultures of *Phytophthora paeoniae* Cooper & Porter about the same when exposed for 4 days to various wave lengths and the full spectrum of visible light. No conidia or oospores were produced under red and yellow-orange filters, though they appeared abundantly under all others.

The biological effects of Hertzian rays is a comparatively new study. It is known that physiological changes in higher organisms may be brought about by the action of short Hertzian waves, but nothing has been recorded of their action on pure cultures of fungi. In week-old albino rats, death occurs, accompanied by a violent rush of blood to the fore and hind limbs and tail, when exposed to an electrostatic field of 100,000,000 cycles per second (13). The degree of external heat necessary to duplicate the effects

was found to be not less than 160° C., which is much higher than any measurable heat generated in the electrostatic field.

EXPERIMENTAL WORK

a. *Gamma rays.* The source of gamma rays used in the experiments was approximately 1 mgm. of radium bromide, sealed in a small glass tube. The alpha particles given off in the disintegration activity of the radium were undoubtedly stopped by the walls of the tube, and, while there was most probably a certain amount of beta radiation through the glass, it could not have been very intense because of the absorption in the glass and the small amount of radium.

The cultures in each case were exposed 4 at a time, by placing them vertically in a tin box with the radium between the 2nd and 3rd cultures. Four controls were shielded by lead, half an inch in thickness, from those being irradiated.

After continuous irradiation of *Sclerotium bataticola* for 72 hours, there was essentially no difference, either in total average areas attained by the mycelium, or the appearance of the cultures, between the irradiated and controls. The same was true with the acidity of the medium adjusted to a pH of 4.6 instead of 6.01. When, however, cellophane was substituted for the glass cover of the Petri dish immediately below the radium, there was a marked suppression of growth, manifested by a much thinner mycelium and delayed formation of sclerotia, which, after a period of 7 days, were far less numerous than in the other 3 irradiated cultures and controls. This effect was most probably due to the action of beta rays, whose intensity had been greatly reduced in the previous cases by the glass cover of the Petri dish but which penetrated the very thin cellophane in sufficient quantity to exert an effect on the organism.

With *Fusarium batatatis,* variations in pigment and sporulation occurred; but, after a number of control experiments, including several that indicated that the radium had no effect on the medium, they were apparently not the result of any action of the rays.

It was possible to use the half-culture method of exposure by cutting out plates of lead to shield $\frac{1}{2}$ of the Petri dishes, but no difference between the exposed and nonexposed portions of the cultures was observed after irradiations of 2 weeks' duration, from the time of inoculation, except where cellophane was substituted for the glass cover, as in the preceding organism. When this was done, the mycelium towards the radium was at first greatly retarded in development but later began to grow, with a large production of rhizomorphic strands (Fig. 1, A).

The half-culture method of exposure was used entirely with *Collybia dryophila.* The growth of this organism was suppressed in the part closest

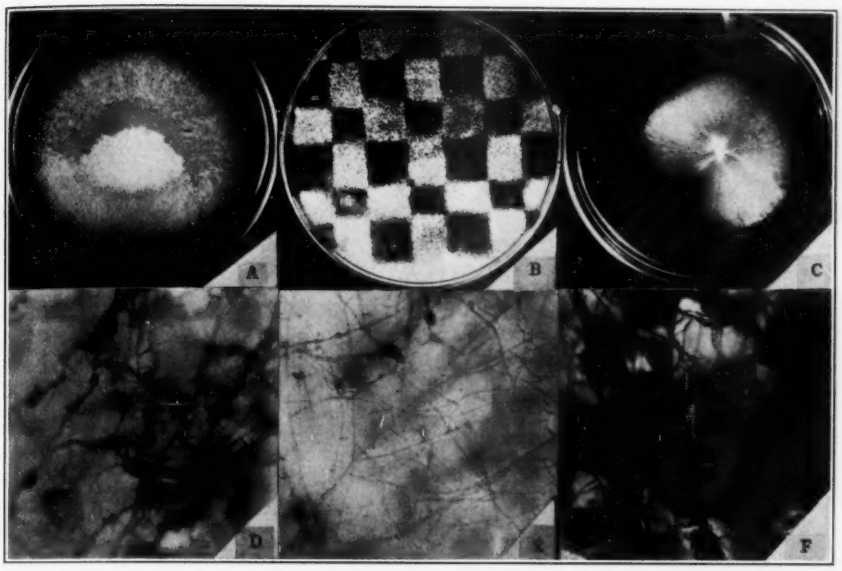

FIG. 1. A. Result of exposing ½ of culture of *Fusarium batatatis* to a glass tube containing radium, through cellophane cover, 9 days, beginning immediately after inoculation. Upper half shielded with a lead plate. The slight suppression of growth and increase in rhizomorphic strands in the lower half, probably a beta-ray effect. B. Culture of *Sclerotium bataticola* irradiated by ultra-violet light through a cellophane cover, 15 min. a day for 10 days, 8 in. from the lamp. Alternate areas of culture exposed to action of light by painting black squares on cellophane cover before first irradiation. Development of mycelium and sclerotia in exposed portions, represented by light squares; weak in comparison to those in shaded areas. (Photographed by transmitted light). C. Suppression of development of the mycelium of *Collybia dryophila* in part of culture nearest glass tube containing 1 mgm. of radium bromide. Probably a gamma-ray effect. D. Photomicrograph of the mycelium *in situ* in a culture of *Sclerotium bataticola* irradiated 20 min. daily for 4 days, 8 in. from ultra-violet lamp beginning 2 days after inoculation. E. Same as D, showing appearance of mycelium at surface of medium. F. Photograph of mycelium in same culture, taken about 2 cm. from that in D and E, showing normal development in portion of culture shielded from rays by a lead plate.

to the radium (Fig. 1, C). The inhibition took place only in the culture immediately above the radium, and, though it was slight, it must have been a gamma-ray effect. The failure to affect the culture below the radium could be accounted for by the slightly greater distance from the source of rays, whose intensity, of course, varies inversely as the square of the distance. The culture medium intervening between the radium and the organism that was inhibited would absorb a negligible amount of the very penetrating gamma rays.

With cellophane in place of the glass Petri dish cover, there was a complete inhibition of development. After 11 days the bit of mycelium used

to inoculate the culture had turned a brownish purple and was apparently dead, but, when removed from the radium, it soon began to develop and the new growth took on the appearances of the normal fungus.

b. *X-rays.*—Although about 500 cultures of the 3 species were experimented on, no modification was found that could be attributed to the action of X-rays. In some of the experiments on *Sclerotium bataticola* there was, apparently, a slight stimulation in the average rate of growth of irradiated over control cultures, but it was always so little as to be well within range of experimental error and the natural variability of the organism. In other experiments, under the same conditions, the average growth rate of the controls was slightly greater, so the net statistical results of a large number of cultures just about balanced. The half-culture method of exposure, with the aid of lead plates, was used as well as exposure of whole cultures with separate controls.

Except for a few preliminary experiments, cultures were all irradiated directly under the cathode. Table 1 gives a list of the conditions of exposure. An average of 16 cultures, with as many controls, was used in each experiment, and several experiments were repeated sufficiently to indicate that there was, most probably, no variation due to the X-ray treatment.

c. *Schumann region of the ultra-violet.*—In the present study a technique that takes advantage of the transparency of nitrogen to Schumann rays was used. The apparatus was constructed according to the following description.

To several 1-liter Pyrex Erlenmeyer flasks a short but wide glass tube that could be stuffed with cotton was sealed near the bottom. Short glass rods were sealed to the inside of each flask, about 1 cm. from the bottom and projecting horizontally from the sides, so that they could furnish a support for a pasteboard shelf, cut in a semicircle to extend halfway across the flask. Into the mouth of the flask was fitted a 4-hole rubber stopper, 2 holes occupied by heavy copper rods that extended down to within $3\frac{1}{2}$ inches of the bottom and the other 2 by glass tubes to allow an inflow and outflow of nitrogen. The inner ends of the copper rods were bent close together to serve as a spark gap. The glass tubes on the outside were bent in a double "S" to prevent spores and bacteria from drifting into the flask. The tube for the inflow of nitrogen extended down to the pasteboard shelf on the inside, and the outer end was adapted to fit into a 2-hole rubber stopper to a wide-mouth bottle containing a mass of loose cotton. The flask and wide-mouth bottle with cotton were sterilized by hot air, while the rubber stoppers, with glass tubing and copper rods assembled, ready to be fitted into the flask and bottle, were wrapped in gauze and sterilized in the autoclave. A sterile culture medium was then poured into the flask to a depth of 1 to

TABLE 1.—*Conditions of irradiation by X-rays of the 3 fungi studied, causing no variation in the organisms that could be attributed to the action of the rays*

Organism	Time of exposure, in number of hours after inoculation					Length of exposure each time	Total length of exposure	Distance from cathode	Kilovolts	Milliamps.	Shortest wave length in A. u.
	1st	2nd	3rd	4th	5th						
Sclerotium bataticola	1	56				2 secs.	4 secs.	79 cm	65	30	.207–.177
	1	56				4 "	8 "	" "	"	"	" " "
	1	56				8 "	16 "	" "	"	"	" " "
	1	48				14 "	28 "	31 "	"	5	" " "
	1	48				42 "	84 "	" "	"	"	" " "
	1	48				252 "	504 "	" "	"	"	" " "
	1	48				10 mins.	20 mins.	" "	"	"	" " "
	24	48	56	72	96	10 "	50 "	" "	"	"	" " "
	24	48	56	72	96	15 "	75 "	" "	"	"	" " "
	36					9 secs.	9 secs.	46 "	120	20	.103
Fusarium batatatis	1	48				2 secs.	4 secs.	79 cm	65	30	.207–.177
	1	48				4 "	8 "	" "	"	"	" " "
	1	48				8 "	16 "	" "	"	"	" " "
	72	104				10 mins.	20 mins.	31 "	"	5	" " "
	72	104				20 "	40 "	" "	"	"	" " "
Collybia dryophila	192					40 mins.	40 mins.	27 cm.	65	5	.207–.177

2 mm., the stoppers fitted into place, and the whole apparatus sterilized again in the autoclave. Several of such units were used in making the cultures to be studied. Inoculations were made through the tube near the bottom of the flask, after which it was closed with a rubber stopper.

As soon as the development of the mycelium was sufficient to allow one side of it to be well shielded by the pasteboard, irradiation was accomplished by means of a copper spark in an atmosphere of nitrogen. While it would not be possible to state the lowest limit of the waves that reached the organism, it is pretty certain that they extended well into the Schumann region. Nitrogen is transparent to Schumann rays, and, although the absorption increases regularly with decrease in wave length, it is very little as far down as λ1250. (12). Data on water vapor are lacking, but there seems to be a maximum of absorption between λ1700 and λ1600, with a possibility of transparency on either side of this region. (12). The absorption of oxygen is very great for wave lengths below λ1850, and, as it was impossible to get out quite all of the oxygen and the presence of water vapor was unavoidable, it is rather doubtful if any of the waves as short as λ1250 reached the organism.

Nitrogen was passed into the flask for at least 10 minutes before irradiation was begun. In order to help remove the small amounts of oxygen present as an impurity in the nitrogen,[3] it was bubbled through 3 bottles of pyrogallol connected in series with the tank of compressed nitrogen and the bottle of sterile cotton by glass tubing. A transformer was employed to step up the ordinary lighting current of 110 volts to about 12,000, which gave a spark producing a very intense ultra-violet spectrum. It was found that the heat from the spark was not sufficient to cause a rise in temperature of 2° C. in 5 minutes in the culture below it. When a total irradiation of more than 5 minutes was made it was made intermittently, so that heat effects would be sure to be avoided.

Because of the time-consuming nature of the experiments with the Schumann rays, it was not possible to study as many cultures as with the others. The results that were obtained, however, were consistent with those described by Bovie (3).

A three-day-old culture of *Sclerotium bataticola* was irradiated for a total of 20 minutes. When a microscopic examination of the mycelium was made 4 hours later, striking differences were revealed between the control and irradiated sides of the culture. The protoplasm in the cells of the mycelium that had been irradiated was discontinuous, had a coagulated appearance, and was much more granular than the control. Towards the periphery the mycelium was composed chiefly of long, straight hyphae, with

[3] This method is not, of course, very efficacious for purifying nitrogen, but apparatus for burning out the oxygen was lacking. Small amounts of oxygen that reached the flask were probably consumed at once by the copper spark.

very few side branches; while, in the shielded area, the side branches were numerous, which would seem to indicate that during the 4 hours intervening between the experiment and the examination, little or no growth had taken place in the irradiated portion. Subsequent experiments showed that it was very difficult to kill a whole area of mycelium by these rays, probably because they are so easily absorbed that they do not penetrate deeper than the uppermost cells. Older mycelium was found much less susceptible to the injurious action of the rays, but essentially the same phenomena occurred to a less degree after exposures of 20 minutes on cultures over a week old. There were always a collapse and withering of the aerial mycelium, effects that occurred to some extent in short exposures of 4 or 5 minutes.

Since *Fusarium batatatis* does not grow so fast as *Sclerotium bataticola,* 5-day-old cultures were the youngest that were irradiated. Ten minutes was found sufficient to cause a collapse of the aerial mycelium and an increase in pigment that became apparent within 24 hours. The mycelium was not entirely killed, however, by exposures of 25 minutes, for new growth would take place within 2 days out from below the superficial layer. Microscopic examination of the superficial portion of the irradiated mycelium revealed a very granular protoplasm, and the granules were relatively large. The most striking effect was the formation of numerous bulbous enlargements at various points along the cells of the mycelium. Sporulation of both micro- and macrospores was abundant in both irradiated and non-irradiated portions but considerably more abundant in the former. A number of examples could be found in the irradiated area of spores on the ends of hyphae. In many cases there was a partial constriction, as if they had started to break off and had been killed or as if sporulation had been induced and, in these cases, was incomplete.

Eight-day-old cultures were found less susceptible to injury than those only 5 days old; but, as in the preceding species, essentially the same phenomena occurred, to a less degree, after exposures of 15 to 25 minutes.

Collybia dryophila grows more slowly than *Fusarium batatatis;* so, it was necessary to wait a week after inoculation before the mycelium had developed to an extent that allowed good shielding of half of it for a control. An irradiation of 20 minutes on 7-day-old cultures arrested the growth on one side, for a period of about 3 days, after which new mycelium would develop out from under that which had been irradiated. Two exposures of 20 minutes each, a week apart, sufficed to kill the mycelium, a fact proved by inoculating new cultures from it. Normal development proceeded in all new cultures inoculated from the shielded side, but no growth took place in those inoculated from mycelium that had been irradiated a total of 40 minutes. Twenty-minute irradiations caused the organism to pigment with a brownish color, separated by a sharp line from the control

side. The surface of the irradiated side took on a smooth, powdery appearance, probably due to the collapse of aerial hyphae. The most striking macroscopic differences were those in size and color of the 2 portions of the culture. Microscopic examination after a 20-minute exposure revealed phenomena similar to the preceding cases in that there were a coagulation of the protoplasm of the overlying cells and the formation of rather large granules.

By using ½ of the same culture as a control in the manner just described, there was a maximum uniformity of conditions, with the light as the only difference; so, there can be little doubt that the results were brought about by the action of the rays, alone.

d. *Ultra-violet rays.*—The source of the ultra-violet light used in the following experiments was a Hanovia Universal Laboratory Model Ultra-Violet Lamp, operated on direct current, 110 volts and 5 ampères. An electric fan was placed where it could constantly ventilate the quartz tube of the lamp. By this means overheating of the tube was avoided and an additional advantage was secured, for, with the tube kept at a constant temperature, the ampèrage through the lamp was found not to vary so much as 0.2 of an ampère during long exposures of over an hour. Irradiations were not begun until the lamp had been in operation a few minutes, in order to secure uniform intensity of illumination. To get the most nearly vertical rays irradiations were never made on more than 4 cultures at a time. Inasmuch as the half-culture method of exposure was used almost entirely, the parts of the organisms to be irradiated could always be exposed directly under the quartz tube. The part of the Petri dishes on the side away from the tube was covered by a lead plate.

In order to utilize the shortest wave lengths given off by the lamp and at the same time maintain the purity of the cultures, a new technique of irradiation was devised, embodying the substitution of cellophane in place of the glass cover of the Petri dish (11). The spectrograms (Fig. 2) of the lamp used in the experiments give the transmission of the cellophane as well as the filters that were used to cut off the shorter wave lengths. Cellophane transmits the full range of the light given off by the lamp with practically no absorption apparent from the photographs of the spectrum. Spectrograms taken on a spectroscope focussed for the short wave lengths showed that the lowest limit of the waves given off by the lamp was actually around $\lambda 2000$. Photographs of a copper spark, on the same spectroscope, taken through the various filters, showed that the actual limit of transparency extended to lower wave lengths than are registered in figure 2 from the mercury-vapor lamp. The practical limit of transmission in each case may be considered as follows:

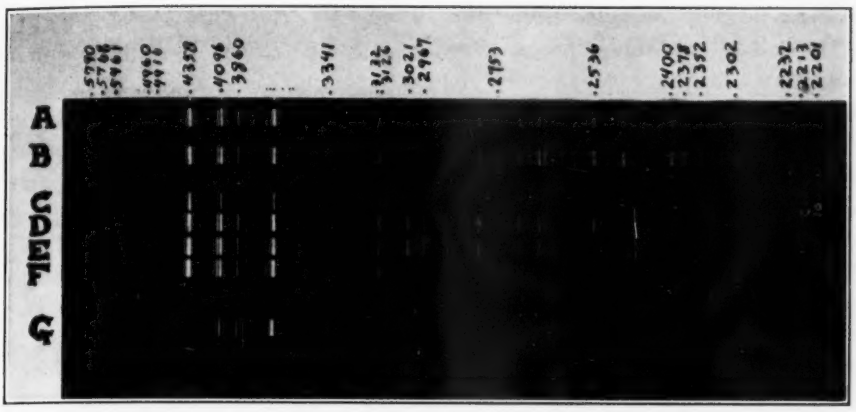

FIG. 2. The mercury-vapor spectrum of the lamp used in the experiments, with the transmission of the different filters. (Some of the lines were lost in the printing). A. Mercury-vapor spectrum, no filter, λ5790 to λ2201. B. One thickness of cellophane, λ5790 to λ2201. C. Culture medium, 2.5 mm. in thickness, λ5790 to λ2536. D. Filter I (Corning 980 A), λ5790 to λ2302. E. Filter II (Vitaglass), λ5790 to λ2536. F. Filter III (Corning G 86 B), λ5790 to λ2907. G. Filter IV (Corning G 585 A), λ4096 to λ3126.

Filter I (Corning 980 A) λ2302; filter II (Vitaglass) λ2536; filter III (Corning G86 B) λ2907; filter IV (Corning G 585 A) λ4096 to λ3126.

To test out the sensitivity of each species to the lethal action of the rays 3 experiments were done in a similar manner. Into each of 25 test tubes, 5 cc. of distilled water and 10 lead shots were put. The test tubes were plugged with cotton and autoclaved. When cool a small piece of mycelium bearing agar was introduced from a pure culture into each test tube. By shaking thoroughly, the mycelium was broken up, and, in the case of *Fusarium batatatis,* the spores disentangled from it. The liquid in the test tubes was then poured over the surface of stiff agar culture medium in Petri dishes, cellophane covers applied, and the newly inoculated cultures irradiated at once for periods ranging from 1 to 50 minutes, 8 in. from the lamp. Five cultures were reserved as controls. With each species, there was hardly any difference in the subsequent development of exposed and unexposed cultures. All germinated normally, and there was abundant germination from spores of *F. batatatis,* even in the cultures irradiated 50 minutes. The spores were not covered by as much as 1 mm. of water. The discrepancy between this and the results of other investigators, who have recorded lethal effects on spores following irradiations for intervals as short as 1 minute (9), seems rather too large to be accounted for only by the differences in sensitivity of different species. The same results were obtained in the present experiments when the cultures were exposed to the

direct action of the rays without the cellophane covers. The intensity of the light is, no doubt, an important factor and may account to some extent for the rather large discrepancies. Accurate comparisons cannot be made between the work of different authors without a record of the intensity, as nearly exactly as possible, of the light used in their experiments. Furthermore, it might be well to point out here that comparisons of the action of different wave lengths, where glass filters are used to eliminate certain portions of the spectrum, are of little significance without a knowledge of the percentage transmission of each filter, and a regulation of the amount of the light reaching the organism, so as to be equal. For a comparative study of several species, however, when the same filters are used in all the experiments, the results are qualitatively perfectly good.

For the remainder of the experiments[4] the half-culture method of exposure was used entirely, except in one case (Fig. 1, B), and, mostly, according to the following scheme of varying the intensity of the light, by distance and length of exposure, as follows:

8 inches, 5 minutes; 8 inches, 10 minutes; 16 inches, 5 minutes; 16 inches, 10 minutes.

Cultures were irradiated after the mycelium had grown to an area of about 2 cm. in diameter. This was attained in *Sclerotium bataticola* from 36 to 48 hours after inoculation; in *Fusarium batatatis*, 3 to 4 days; and in *Collybia dryophila*, 5 to 6 days. Irradiations were generally made every 24 hours for a period of 5 days.

Under cellophane, alone, 5 minutes' irradiation a day, at 16 in., was sufficient to cause a noticeable suppression of growth in *Sclerotium bataticola*, with an increase in pigment in the mycelium and a tendency for it to grow deeper into the medium. These reactions occurred to a greater extent with longer exposures at less distance from the lamp. (Fig. 1, D, E, and F.) There was an inhibition of the formation of sclerotia, along with the increase in pigment, but sclerotia would continue to form slowly in parts of cultures irradiated daily for 15 minutes, at 8 in. Figure 1, B, shows very clearly how the action of the light inhibited sclerotial formation, and, although it is not apparent in the photograph, this culture demonstrated particularly well the manner in which the mycelium grew deeper into the agar under the influence of the rays. In the black squares, which represent the shaded portions of the culture, the mycelium continued to develop until these areas were over 2 mm. higher than the light squares, which represent the irradiated areas, in which there was scarcely any mycelium on the surface of the medium.

[4] Control experiments, in which half-portions of the medium in Petri dishes were irradiated before inoculation, indicated that the light did not bring about reactions in the medium that might affect the organisms.

Under filters I and II, cutting off wave lengths below λ2302 and λ2536, respectively, there was very little difference between the two sides of cultures irradiated daily for 10 minutes, at 8 in. There was a slight suppression of the aerial mycelium, and an increase in pigment that was never very striking. The variations were usually most noticeable after the third exposure and to a greater extent under filter I than filter II but were generally blotted out, so to speak, by the rapidly growing mycelium after the fifth exposure, when the cultures were a week old. This effect would seem to indicate that the young mycelium is more sensitive to the rays than the older, a fact which might account, to some extent at least, for the apparently great resistance to the 50-minute exposures in the first experiment.

There was no effect under filter III, which cuts off the rays below λ2907, of filter IV, transmitting between λ4096 and λ3126, after irradiations as long as 2 hours and 15 minutes at a distance of 8 in.

With *Fusarium batatatis*,[5] as with the preceding species, the most pronounced reactions occurred under cellophane, alone. Under filters I and II, the same types of reactions occurred, to a less degree, as under cellophane. The effects of the light, under these filters, were much more prominent with this species than with *Sclerotium bataticola*. Daily exposures of 5 minutes, at 8 in., caused a very pronounced increase in pigment and a very noticeable suppression of growth. (Fig. 3, A and B). Semicircular rings appeared in the mycelium at points corresponding to the extent of its development at the time of each exposure. This was an interesting phenomenon because it occurred only under certain conditions in each of the 3 organisms studied. It was correlated with at least two things: (1) the natural growth rate of the organism and (2) the filter under which it was irradiated. With *F. batatatis* they were most prominent under filter I but were noticeable under all of them whenever the length of exposure was sufficient to produce a reaction. With *S. bataticola* they occurred only under cellophane and with exposures of between 5 and 20 minutes daily at 8 in. or longer exposures at 16 in. There never appeared more than 3 rings in this species because they always appeared at a point corresponding with the extent of the mycelium at the time of irradiation, probably because the youngest mycelium was the most sensitive to the action of the rays, and within 3 days after the first irradiation the mycelium of this organism had covered the surface of the medium in the Petri dish. With *Collybia dryophila* the rings did not form at all under cellophane but occurred under both filters I and II whenever the exposure was long enough to produce any inhibition of growth. An explanation for this phenomenon will be suggested farther on in the discussion.

Under filter II, the only reactions that occurred in *Fusarium batatatis* after weak exposures, such as 5 minutes a day, at 16 in., were a very slight

[5] Differential counts of sporulation were not made in this study.

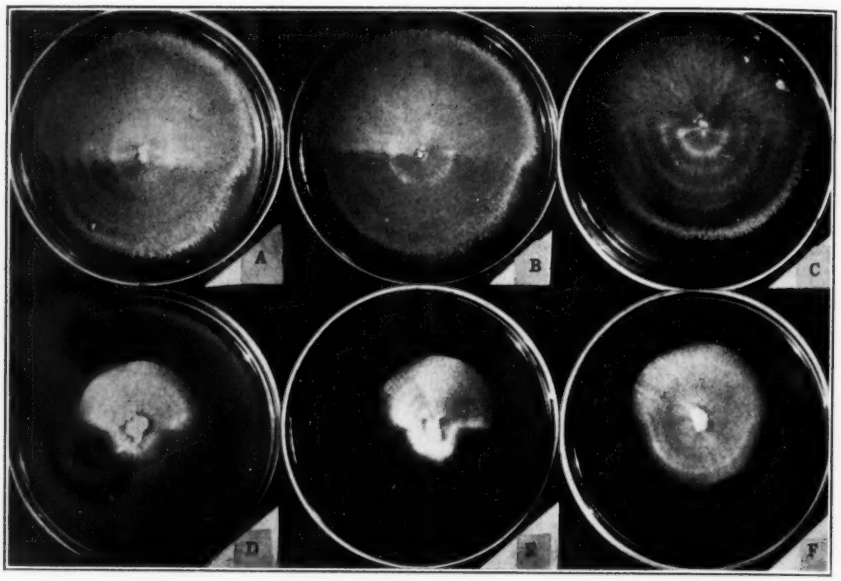

FIG. 3. A. *Fusarium batatatis*, after exposing lower half of culture to full range of ultra-violet light through cellophane cover, 10 min. a day, for 5 days, at 8 in. from the lamp, beginning 3 days after inoculation. B. Same as A, except that exposures were only 5 min. a day. C. Same as A, but with filter I, cutting out rays below λ2302, covering exposed part. D. *Collybia dryophila*, after exposing lower half to full range of ultra-violet light through cellophane cover, 10 min. a day, for 5 days, 8 in. from lamp, beginning 5 days after inoculation. E. Same as D, except exposures were for only 5 min. a day. F. Same as D, but with filter I, absorbing rays below λ2302, covering part of culture exposed to rays.

increase in pigment and very faint formation of rings. The reactions under filter I were always greater for similar conditions of exposure than under filter II. Under filters III and IV no variation occurred after exposures as long as 3 hours at 8 in.

With *Collybia dryophila* there was a pronounced suppression of growth after exposures of 5 minutes daily under cellophane at a distance of 8 in. (Fig. 3, D and E). There was no increase in pigment, although it will be recalled that such an effect occurred in the experiments with Schumann rays. The inhibition in growth was not so great as the photographs might indicate, for, when the cultures were held up to the light, it could be clearly seen where development of the mycelium had taken place under the surface of the medium. Under filters I and II, especially the latter, the inhibition was much less striking (Fig. 3, F). It is interesting to note in this connection that the 2- to 3-mm. thickness of culture medium has approximately the same lower limit of transmission of the rays as filter II, so the development of the mycelium deep in the medium is readily understandable. When the

daily exposures were discontinued and the culture set aside the underlying mycelium would soon return to the surface and continue to develop normally at a point about 2 mm. beyond where it was, below the surface, at the time of the last exposure.

As with the preceding species, there was apparently no effect at all under filters III and IV, after exposures as long as 3 hours at 8 in.

Returning to the phenomenon of ring formation under the action of the light, it has already been stated that this was correlated with the filter used and the natural growth rate of the organism. From the observations made in the present study the mechanism for this phenomenon, which is no doubt partly in adaptation to injurious action of the rays, depends on the alternate development of the mycelium deeper in and, then again on, the surface of the medium. This depends, first, on whether the action of the rays is strong enough to cause the mycelium to grow into the medium, in spite of other adaptive responses, such as pigmentation, which may take place in resistance to the light. The wave lengths transmitted by cellophane were obviously those that produced the greatest effect, while filters I and II, successively, absorbed more of the wave lengths capable of bringing about ready response in the organisms. Bearing these facts in mind, together with the fact that when rings appeared, at all, they appeared at points corresponding with the extent of the mycelium at the time of the last irradiation, an explanation for the formation of the rings in the cultures as described above suggests itself at once. They appeared in *Sclerotium bataticola* only under cellophane, alone, because the other filters absorbed the waves capable of causing the mycelium to grow deeper into the medium. This organism grows so fast that, in the 24-hour interval between exposures, the mycelium returned to the surface where development continued rapidly until the next irradiation, which slowed it up and sent it down into the medium again. *Collybia dryophila* showed no signs of rings under cellophane, alone, because it grows so slowly that after an initial irradiation sufficient to cause it to grow into the medium, there was not enough time in the 24-hour interval between irradiations for it to return to the surface and undergo any considerable development there. Rings appeared in this organism under filters I and II because these filters cut out the more effective rays and caused only a partial growing of the organism into the medium. The mycelium was not affected to the extent that it could not continue developing well on the surface. *Fusarium batatatis,* which has a growth rate about midway between those of the other two fungi, showed the formation of growth under cellophane and filters I and II because the rays transmitted by each had an effect sufficient to cause the mycelium to grow into the medium, while the growth rate was fast enough to allow new development on the surface during the interval between exposures. The rings were

most prominent under filter I because the wave lengths transmitted by cellophane exerted an effect great enough partially to blot them out, so to speak, while the wave lengths transmitted by filter II did not exert an effect sufficiently great for them to become very prominent. While this hypothesis for the cause of ring formations, as described, is evidently true, from observation of a large number of cultures, it is not offered as the whole story underlying the formation of rings in the present study nor as an explanation for the occurrence of such structures in pure cultures of fungous organisms in general. Factors other than those of natural growth rate of the organism combined with the action of different ranges of wave lengths of the light no doubt enter into the cause of the phenomena, but the correlations described are perhaps interesting for whatever they may be worth.

One thing was very consistently observed in the experiments with these rays, *viz.*, that there was often very little difference in the 5- and 10-minute exposures on an organism at a given distance. It would be practically impossible to obtain accurate numerical data in support of this statement, but it is almost certain that in no case did a 10-minute exposure produce twice the reaction of that of a 5-minute exposure, at the same distance, for it was often hard to tell them apart from the appearance of the cultures. Likewise, doubling the distance did not seem to reduce the effect by anything like as much as $\frac{1}{4}$ for exposures of corresponding lengths of time, although the intensity of the light was reduced by that much.

To determine the relative sensitivity of *Fusarium batatatis* and *Collybia dryophila* to the light transmitted by the different filters, a uniform procedure of irradiation was arbitrarily chosen. The length of the exposure, at 8 in. from the lamp, which was just sufficient to cause a variation in the organism within 24 hours after irradiation, was determined. Irradiations were made when the mycelium had developed to an area of about 2 cm. in diameter. After a few preliminary experiments to find the approximate length of exposure needed, a series of cultures of each organism was irradiated for gradually increasing lengths of time in order to get more nearly exact data. While there was some variability in the lengths of exposures for individual cultures, the results, after repeated experiments, were, on the whole, fairly consistent. The lengths of exposures varied within the limits set forth below:

Filter	*F. batatatis*	*C. dryophila*
Cellophane alone	10 to 15 seconds	1–2⅝ to 2¼ minutes
Filter I	3¼ to 4 minutes	5½ to 6 "
Filter II	4 to 5 "	6¼ to 6¾ "

e. *Visible light rays.* The apparatus for irradiating with visible light rays consisted of a large wooden box, painted black inside and out, that

could be opened from the side in order to put in or remove cultures to be exposed. It was divided into 3 light-proof compartments, with 2 holes, each 4 in. square, cut in the top of each. These openings could be covered by filters transmitting different ranges of wave lengths. Above each hole was a 150-watt globe that was lowered into a glass battery jar supplied with a constant stream of cool water. The water spilled over the tops of the jars into Pyrex pans that were slightly tilted and sealed to a drain by means of beeswax and paraffin. The water kept the globes perfectly cool and absorbed the infra-red rays. The temperature inside the box was seldom found to vary more than 2 degrees on either side of 22° C. The intensity of the light was reduced by having to pass through the water, the glass of the battery jar and Pyrex pan, and the color filter before reaching the organism on the floor of the box 2½ feet below the filament of the globe, but these precautions were found necessary in order to maintain a constant temperature favorable for the growth of the cultures and avoid possible heat effects.

In addition to the full range of visible light, violet, blue, orange, and red color filters were used. None of the filters was quite spectrally pure. The half-culture method of exposure was used entirely, by means of pasting a strip of black paper over half of the top of the Petri dishes.

No positive or consistent variation was noted in irradiated cultures except in one case. When *Fusarium batatatis* was exposed to the full range of visible light for a period of a week or more, starting with the time of inoculation, there was a slight but distinct increase in pigment. Sporulation was abundant under all filters. There was no evidence of any influence on the growth of the mycelium in any of the organisms, after exposures of over 2 weeks beginning right after inoculation. These particular organisms may be naturally more resistant to the action of visible light rays, or perhaps the intensity of the light was not so great as that which caused variations in other organisms described by previous investigators (1), (6), (19).

f. *Infra-red rays.* The source of the infra-red radiations was a 150-watt globe, placed where it could be constantly ventilated by cool air through an open window, 2½ ft. above the sill. Cultures of the organisms were placed on the sill in a flat pasteboard box, with an opening 4 in. square, cut in the top. The opening was covered by a Wratten and Wainwright stained gelatine light filter, transmitting no waves below λ6900 and less than 5 per cent below λ7000. One-half of each of 2 cultures could be exposed at the time under this filter.

The results were entirely negative. There was apparently no effect on any of the organisms after exposures lasting as long as 10 days, starting immediately after inoculation.

g. *Hertzian waves.* The half-culture method of exposure was possible with Hertzian waves by means of wrapping half of the Petri dish with

lead foil to neutralize the field in that portion and exposing the other half between a condenser made of 2 copper plates, 4 in. square. The electrostatic field between the plates oscillated with a frequency that gave a wave length of 100 m. in the first experiment and 50 m. in the second. A UX 210 tube was used in the set.

The results of the experiments with Hertzian waves that have been completed so far have been entirely negative. There was no effect on 3- and 4-day cultures of *Fusarium batatatis*, on 4- to 8-day cultures of *Collybia dryophila*, or 3- to 4-day-old cultures of *Sclerotium bataticola* as a result of constant irradiation for 65 hours by a wave length of 100 m. and for 48 hours by a wave length of 50 m.

DISCUSSION

The present study, covering as it does such a broad range of wave lengths through the electromagnetic spectrum, on 3 different fungi, is by no means an exhaustive one. It seems sufficient to indicate, however, that the action of rays in certain portions of the spectrum are more likely to cause variations than in others. The fungi studied were all sensitive to more or less the same ranges of wave lengths, and the variations that occurred differed more in degree than in kind.

The results were necessarily inconsistent with those of some of the previous investigators, for the review of the literature, especially in the case of the ultra-violet rays, revealed a good many apparently wide discrepancies between the results of different workers (18), (21), (15), (9), (2), (16), (19), (1). On the whole, the discrepancies were more quantitative than qualitative, which is perhaps as much the result of different conditions of experimentation as of differences in the organisms. The rôle of the intensity of the various rays has been little and unsatisfactorily touched upon so far; and, although it is a complicated study, requiring careful and sometimes tedious as well as difficult calibration of instruments in order to secure accurate data, it might yield valuable results. Until such a procedure is adopted, accurate quantitative comparisons between the work of different authors, as well as between the action of different wave lengths, are impossible.

The only rays, in the present study, that were effective in causing variations in the organisms were those in the Schumann and short ultra-violet regions, from λ1250 up to λ2536, although variations might have occurred under the influence of other rays had the intensity been great enough. No effect occurred as the result of X-rays, and only a very slight effect, evidently due to gamma rays, occurred with radium. Inhibition in growth of all 3 fungi occurred with radium, but, except for a slight inhibition in the case of *Collybia dryophila*, the effects were probably caused by

beta rays instead of the electromagnetic gamma rays. Ultra-violet rays caused a suppression of growth, together with reactions that may be considered as adaptive against the injurious actions of the light, while the Schumann rays apparently exerted such destructive effects on those cells of the mycelium to which they penetrated that the organisms could not undergo adaptive responses in resistance to their action. The problem of adaptive responses is an interesting one, and it is here suggested that such responses, manifested under the influence of the ultra-violet light in such ways as the increase in pigment and growing of the mycelium deeper into the medium, may be responsible for the evident failure of the organisms to react in direct proportion to the length of the exposure or inversely to the square of the distance from the source of the rays. It is, in the first place, necessary to bear in mind that in working with living organisms a whole series of complicated reactions may come in to upset, to a certain extent, the exact conformity to physical laws that hold for less complex bodies. Assuming that the action of the short ultra-violet rays is of an injurious nature, as it evidently is, the natural *adaptability* of the organism to the light is suggested as one of the factors that may throw the variation slightly out of proportion with the intensity of illumination and length of exposure. The *adaptability* of the organism is little more than a name for a number of reactions that may take place entirely unobserved within the organism or become evident through variations in pigment and the methods of growing into the medium. Under a given set of conditions there is probably a definite threshold for the amount of light necessary to make a variation become evident in the organism. The threshold of a given organism would undoubtedly vary under different conditions, depending, for example, on whether the irradiation was by light of great or less intensity and on the age of the culture. If there were a very accurate way of securing numerical data on this subject, the thresholds would be a good way of determining the relative sensitivity of different organisms to various wave lengths. In working out the minimal lengths of exposure necessary to cause a variation within 24 hours, under a uniform set of conditions, for *Fusarium batatatis* and *Collybia dryophila*, the results were consistent enough to show clearly that a minimum amount of light is necessary for a variation to become evident, and above this the variations occurred comparatively rapidly. It is interesting to note that the threshold for *C. dryophila*, which produces a white mycelium, was higher in each case than that for *F. batatatis*, which is pigmented and may become heavily so under the influence of the rays.

The fundamental nature of the reaction of the organisms to various wave lengths can hardly be conjectured at the present stage of biophysical knowledge. Wave length is evidently of great significance, and apparently

a small intensity of some wave lengths exerts a considerable effect, while other wave lengths might cause no reaction, even though of great intensity.

SUMMARY

1. A study of a total of over 1,500 cultures of 3 fungi was made under the influence of gamma rays, X-rays, Schumann rays, ultra-violet rays, visible light rays, infra-red rays, and Hertzian waves.

2. *Collybia dryophila*, *Fusarium batatatis* and *Sclerotium bataticola* were found more susceptible to variation under the influence of the rays in some portions of the spectrum than in others.

3. A slight inhibition of growth in the mycelium of *C. dryophila* occurred in the parts of cultures closest to the tube of radium. This was evidently a gamma-ray effect. The inhibition of growth in cultures of all 3 organisms, which occurred to the greatest extent in *C. dryophila*, whose development was completely arrested, when exposed to the radium through cellophane covers, was probably a beta-ray effect and hence not due to electromagnetic waves.

4. No variation was observed under the influence of X-rays.

5. Schumann rays were the most destructive but did not affect so large a proportion of the mycelium as the ultra-violet rays proper, probably because the former are so easily absorbed that they did not penetrate deeper than the uppermost cells. In all 3 organisms, Schumann rays caused an apparent coagulation and granulation of the protoplasm in the overlying cells of the mycelium.

6. Ultra-violet rays had a greater or less effect, depending on whether irradiations were made through cellophane, which transmits the full range of the spectrum from a quartz mercury-vapor lamp, or filters that cut off the shorter wave lengths. The rays passing through Corning filter 980 A, cutting off wave lengths below λ2302, had less effect than those through cellophane, which extended down to around λ2000, but exerted a greater effect than the rays passing through vitaglass, which transmitted down to λ2536. An increase in pigment, together with a suppression of growth, occurred in *F. batatatis* and *S. bataticola*. *Collybia dryophila* underwent a suppression of growth but no increase in pigment. The formation of rings in the mycelium of the organisms, under the influence of the light, was correlated with the natural growth rate of the organism, and the wave length and intensity of the light. The responses of the organisms did not seem in strict proportion to the length of the exposure or intensity of the light, possibly because adaptive reactions of the organisms may slightly displace the physical laws holding for less complex bodies. Under an arbitrary set of conditions, a somewhat variable 'threshold'' was found, up to which no effect of the radiations could be observed in *F. batatatis* and *C. dryophila*.

7. The only effect of various wave lengths and the whole range of visible light was an increase in pigment in *F. batatatis* after exposures of a week or more to the full range.

8. No effect was found as the result of infra-red rays above $\lambda 7000$.

9. No effect was produced in the organisms by Hertzian waves of 50 and 100 m., respectively.

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TRANSMISSION OF THE PINEAPPLE YELLOW-SPOT VIRUS BY THRIPS TABACI[1]

MAURICE B. LINFORD

Pineapple yellow spot is a virosis that has risen to prominence recently in the Hawaiian Islands, where it is one of the major diseases of that unusual plant, *Ananas sativus* L. The writer, in a preliminary note (5) has set forth his findings that the most important vector, if not the only one, operating in the field occurrence of this disease, is the onion thrips, *Thrips tabaci* Lindeman. It is the purpose of the present paper to report in more detail the studies upon which that note was based and to present the findings of further studies dealing with the transmission of the yellow-spot virus. The methods of rearing and handling the experimental insects are described in detail because work with thrips presents some special problems not involved with larger insects and because the technique used in this work is quite different from that employed by Samuel, Bald, and Pittman (7) in their work with *Frankliniella insularis*. Other aspects of the disease will be considered in other papers.

FORMER INVESTIGATIONS

Investigations, instituted by the staff of the Experiment Station of the Association of Hawaiian Pineapple Canners shortly after the disease was first seen in 1926, has been in progress continually up to the time the writer assumed his present duties in July, 1929. Although the means of transmission was not then known, much had been learned about the disease that aided subsequent work. A preliminary description of the disease and a partial statement of this earlier work have been prepared by Illingworth (2), formerly Entomologist at this Station. Figures 1 and 2 illustrate the outstanding symptoms.

Before the writer took up this work, the view had already been developed by G. E. Paxton[2] that yellow spot was a virosis. He based this opinion upon his studies of symptoms, stressing the chlorotic rather than the necrotic aspects of the disease and upon the absence of demonstrable microorganisms from the chlorotic tissues. Mr. Paxton, after failing to transmit the disease by various methods of mechanical transfer of the juices of diseased plants, had postulated, furthermore, that this virus required a specific insect vector for its transmission. The fact that the first symptom of this disease is an *initial spot*, which develops characteristically

[1] Technical paper No. 24 of the Experiment Station of the Association of Hawaiian Pineapple Canners, University of Hawaii.

[2] Mr. Paxton reported these findings in the private organ of the pineapple industry.

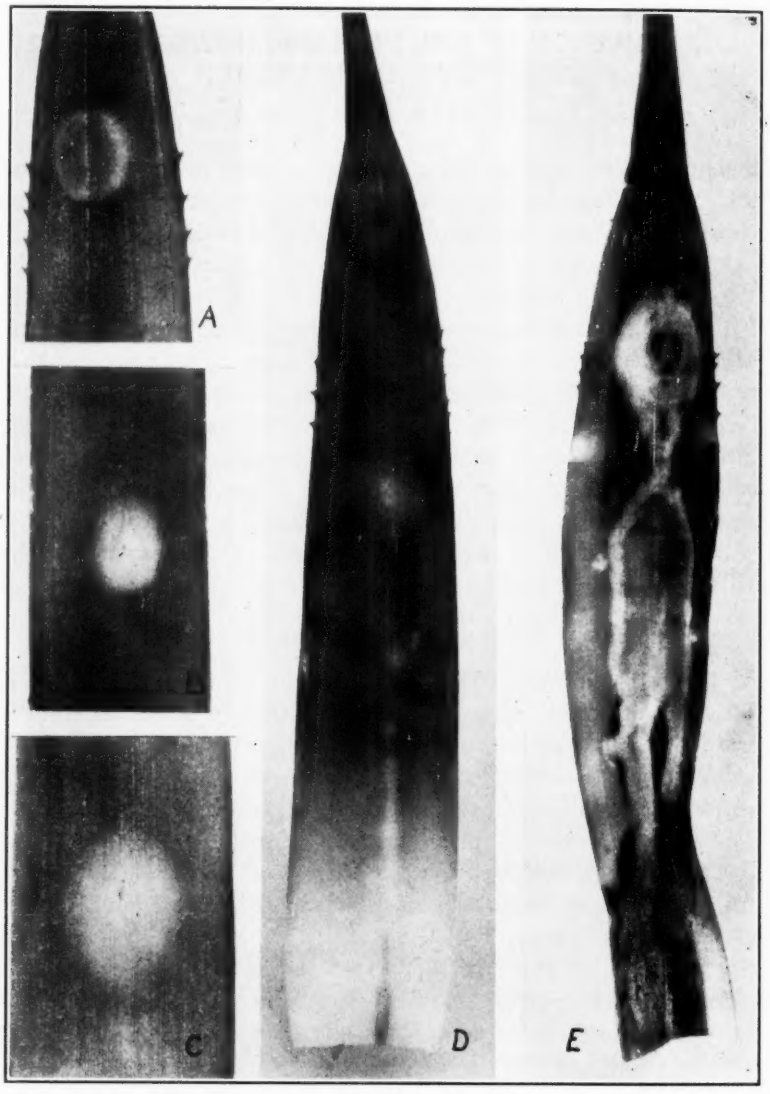

FIG. 1. Initial and later symptoms of yellow spot in pineapple. A, B, and C. Representative initial spots or chlorotic local lesions. D. Somewhat later stage, showing a characteristic initial spot with a yellow streak below it ending in a water-soaked streak at the base of the leaf. E. Later stage of an initial-spot leaf, showing marked necrosis.

a few inches from the base of an elongating leaf, suggested to him that these spots were the points at which the leaf had been inoculated and that the vector was able to inoculate only at or near the base of a growing leaf.

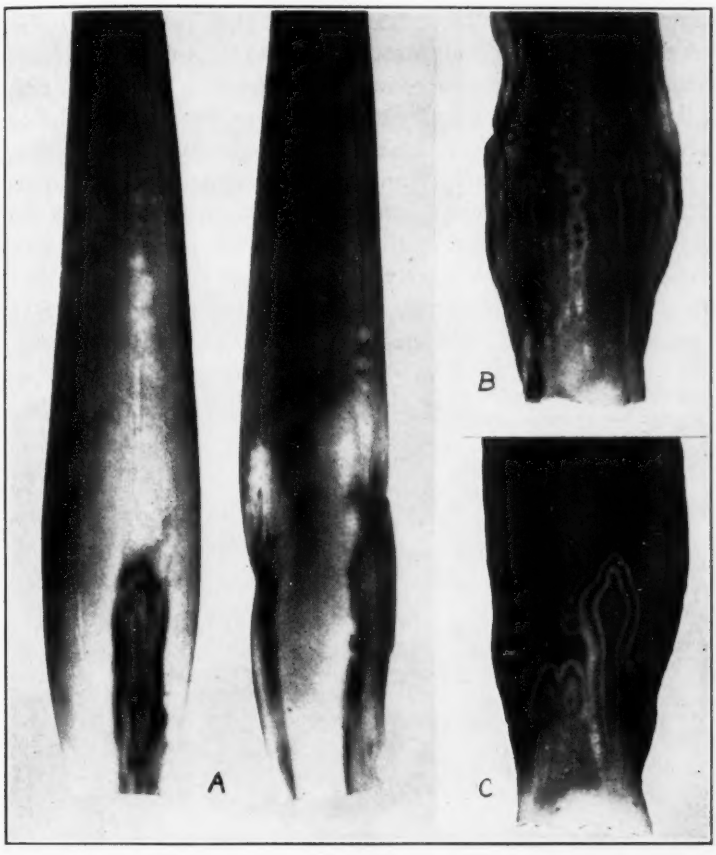

FIG. 2. Pineapple yellow spot in leaves from above the initial-spot leaf. A. Two leaves from a field specimen, showing chlorosis and the necrosis that characteristically follow. B. and C. Unusually elaborate development of chlorotic ring and line patterns on leaves of a seedling plant from the greenhouse.

Elongation of the leaf from its base during the incubation period of the virus in the leaf he held to account for the appearance of the new initial spot several inches up the leaf.

PRELIMINARY STUDIES

Search for the vector. A critical review of the evidence with Mr. Paxton led the writer to accept the above hypotheses as a working basis and to center attention upon the initial spots. Microscopic examination of such spots by R. N. Chapman in the hope of discovering clues as to the nature of the insect that might have fed there revealed the presence of characteristic and minute punctures in the upper surface of the leaf near the initial

spots. Further studies by the writer showed these punctures (Fig. 3, a), which were taken to be feeding punctures made by a relatively large insect, to be almost constantly associated with initial spots. They do not always occur within the spot itself and generally there are none at the center of the spot. They are, however, much more abundant near an initial spot than on other parts of the leaf and are not commonly present on leaves of healthy plants. Subsequent examinations of numerous collections from different islands and at different times of the year have shown these punctures to be associated with nearly every initial spot of the disease. In appearance they have been so uniform throughout as to indicate that they are the work of either a single species or a group of closely related species of insects.

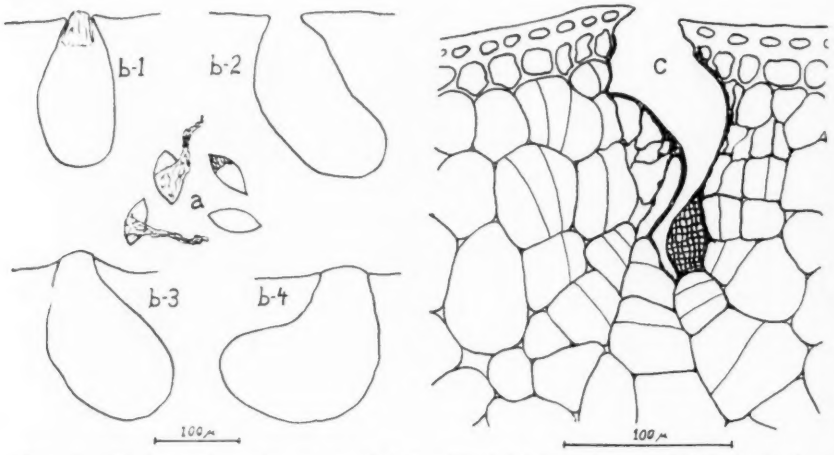

FIG. 3. Thrips ovipunctures associated with initial spots on pineapple leaf. a. Empty ovipunctures as seen from the leaf surface. Note the shrivelled prong that stands up from some of the orifices. This is pulled out by the emerging larva. b–1 to b–4. Relatively fresh ovipunctures from which the larvae have already emerged, as seen in cross-sections of the leaf. Observe that b–1 and b–2 may owe their different shapes to different angles of sectioning. c. An old ovipuncture that is nearly filled up by growth of the surrounding leaf tissue. All drawn with camera lucida. Note that c is enlarged more than a and b.

Microscopic study of sections of the leaf cut through such punctures at different angles (Fig. 3, b) showed that what had been seen from the surface was only the narrow orifice leading to a reniform cavity within the leaf. The shapes of different individual cavities and the conditions of the surrounding cells of the pineapple leaf (cf. Figs. 3, b, and 3, c) indicated that an object had filled the cavity for a time and had then escaped. These studies showed the typical size of the pouch to be about $100 \times 200\ \mu$, as seen in longitudinal section. The finding of egg membranes in some sec-

tions completed the evidence that these cavities were the empty egg-punctures of a very small insect.

Other inconspicuous markings on the leaf surface were then recognized to be probably the feeding marks of the insect that had made the larger punctures with its ovipositor. This feeding injury, like the ovipunctures, was found much more commonly on initial-spot leaves than elsewhere, extending as an irregular line directly through the centers of initial spots.

Of the various groups of insects suggested to the writer by Dr. Illings-

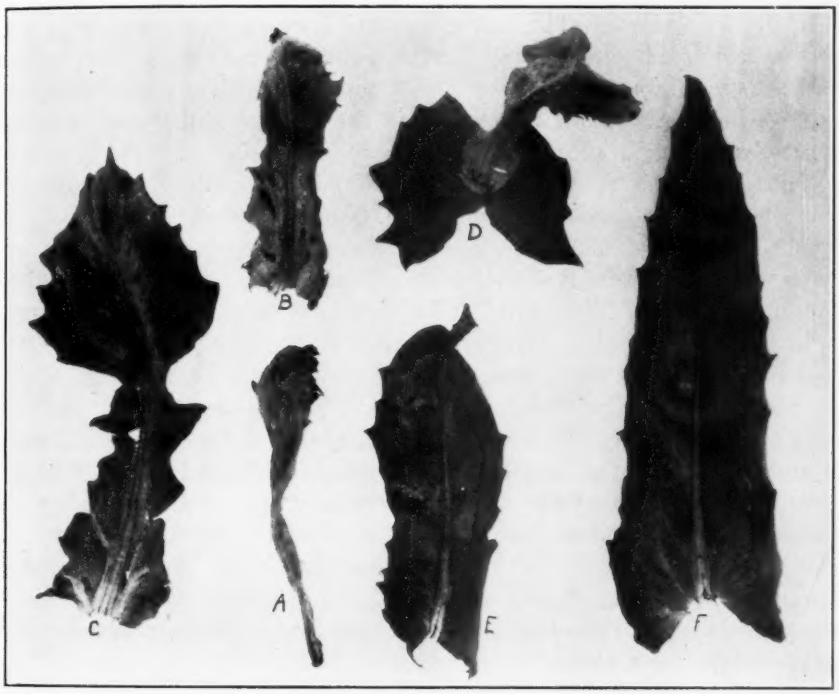

FIG. 4. Yellow spot in *Emilia sagittata* from the field, showing different degrees of mottling and distortion. Normal leaves of this plant are highly varied in shape but all of these leaves except that at the right are somewhat deformed. A is chlorotic throughout; B has a few green islands on a chlorotic background; C, D, and E are partly affected; and F has only a small area slightly mottled near its apex.

worth, only one, the thrips, was able to qualify as to size.[3] Examination of thrips eggs in onion leaves, both in surface and sectional views, revealed striking resemblance in size and form. Finally, pineapple leaves, collected

[3] That *Triphleps* sp., suggested by Illingsworth, was not the vector was shown by the fact that its eggs are much too large to fit specifications. (Illingsworth, J. F. An Anthocorid (Triphleps sp.). U. S. Dept. Agr. Bur. Entom. Insect Pest Survey Bul. 10: 24. 1930. (Mimeographed.)).

by Mr. Paxton from an infested area, were found to contain eggs not yet hatched. These were observed under a binocular microscope and, as they hatched, thrips larvae were seen to emerge from apertures that were typical in all details of the ovipunctures associated with initial spots. Thus it became almost certain that the vector of yellow spot was a species of thrips.

Search for another host plant. Even before the probable vector had been found a search was begun for a weed host of the yellow-spot virus. Evidence from the field occurrence of the disease suggested that whatever the vector might be, it was probably breeding on some infected plant other than pineapple. The suddenness with which newly set fields may become heavily infested suggested a migration of the vector from outside the field, and the standard practices of pineapple culture were such that diseased pineapple plants in near-by fields were too rare to serve as the source of infective insects at the beginning of an outbreak.

Several species of weeds in and around pineapple fields exhibited symptoms of viroses, chiefly of mosaic type, but a disease of *Emilia sagittata* (Vahl) DC.[4] appeared the most closely related to the pineapple yellow spot in symptomatology and in distribution with respect both to space and time. Symptoms on this plant (Figs. 4 and 5) involved, in addition to mottling, a distinct tendency towards ring spot with zonate, circular, chlorotic patterns that suggested the disease in pineapple. Moreover, although Emilia was abundantly distributed over Oahu, it was diseased chiefly in those areas where the pineapple disease was abundant, and was found diseased throughout the range of the pineapple disease on Oahu and Maui.[5] Furthermore, in a field where yellow spot had recently appeared, there were few diseased Emilia plants on October 17, 1929, but the percentage of infected Emilia increased rapidly during the following weeks. For these several reasons, the disease of Emilia was regarded as probably identical in cause with yellow spot and diseased Emilia was used in various experiments during the search for a vector.

This disease in Emilia, which will be described more fully in a later paper, is characterized by extreme mosaic symptoms in the first few leaves produced after inoculation and by a mild circular blotching in later leaves, which sometimes develops into an extremely involved pattern of concentrically zonate lines of dark and light green (Figs. 4 and 5).

[4] This plant was listed earlier (5) under the synonymous name, *Emilia flammea* Cassini, following the usage of Bailey (1). Specimens collected locally were compared with the type of *Cacalia sagittata* Vahl through the courtesy of Drs. Harold St. John and S. F. Blake, with the result that the name *Emilia sagittata* (Vahl) DC. appears to be the correct form.

[5] Since that time it has been found in the diseased condition coextensive with the pineapple disease on 3 islands but not, as yet, on Lanai and Hawaii, where yellow spot is very rare.

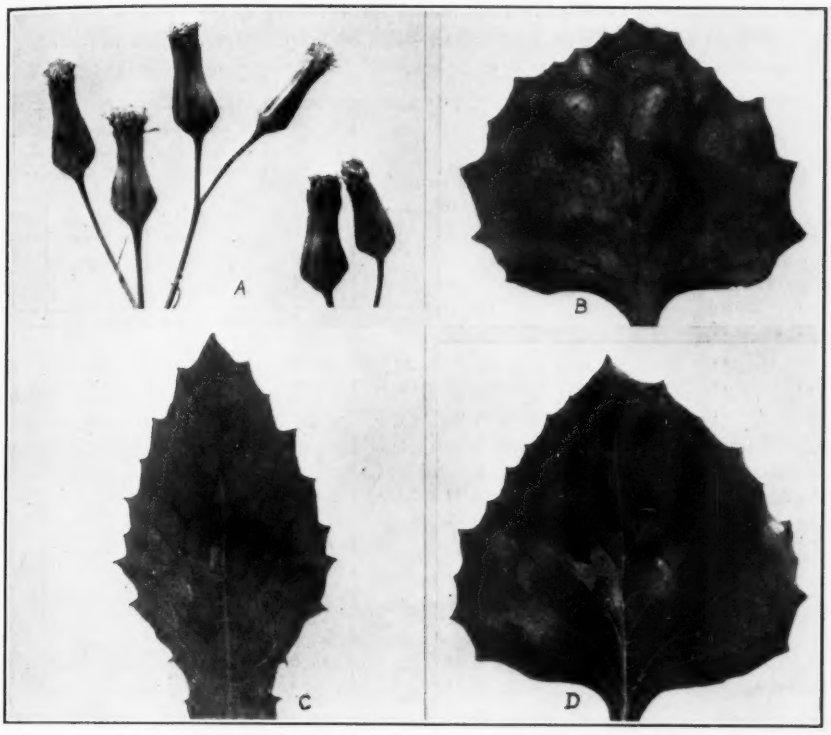

FIG. 5. Varied symptoms of yellow spot in *Emilia sagittata*. A. Mosaic mottling in the form of dark and light green stripes on involucres of flower heads. This develops only immediately following infection. B. Extreme development of zonately banded chlorosis in an old leaf. C. Dark necrotic line extending in zigzag course across the leaf. The line was opaque but not colored. Below it, at the left of the midrib, note a corresponding whitish line. This leaf was below the first mottled leaf on the plant. D. Mild mottling without distortion, a condition often seen on plants in the greenhouse.

Proof that Thrips transmit the virus from Emilia to pineapple. A species of thrips was commonly found on the leaves of diseased Emilia plants. Therefore, when it was learned from the study of insect punctures that thrips were the probable vectors, thrips were collected from diseased Emilia plants in the field and liberated on seedling pineapple plants in individual plant cages in the greenhouse. Table 1 shows the results of these and other preliminary tests. As indicated in the table, 21 of the 50 pineapple plants so treated developed yellow spot. Subsequent tests, some of which are reported in table 2, have shown diseased Emilia plants from the open field to be a reliable source of viruliferous thrips, subject to certain exceptions set forth in the discussion. Moreover, when similar thrips from diseased Emilia were caged upon healthy Emilia, these plants developed

TABLE 1.—*Preliminary inoculation experiments with Thrips tabaci, adults and larvae, collected on various plants in pineapple fields or intercycle areas and liberated on test plants in individual plant insect cages*

Insects		Test plants			
Source[a]	Applied to each plant	Pineapple		*Emilia sagittata*	
		Tested	Infected	Tested	Infected
Emilia sagittata	1 to 10 ad.[b] & l.[b]	29	10		
E. sagittata, diseased	5 ad.	4	3		
" " "	10 "	3	1		
" " "	20 "	10	5		
" " "	5 " & l.	2	1	12	7
" " "	10 " " "	2	1		
" " "	1 to 3 l.			4	3
Emilia sp. (orange), diseased	3 "			1	1
Senecio hieracifolia	1 ad.	4	2	3	2
Bidens pilosa	20 "	10	7		
Radish flowers, *Raphanus* sp.	30 "	10	6		
" " " "	10 "			5	3
" " " "	5 "	5	0		
" " " "	20 "	5	0		
Mustard flowers, *Brassica* sp.	5 "	5	0		
Jack bean, *Canavalia ensiformis*	5 "	6	0		
Pea, *Pisum sativum*	5 "	10	0	32	0
" " "	2 to 4 "			28	0
Sonchus oleraceus	4 l.	1	1	3	1
" "	ad. & l.	3	2	2	2

[a] Thrips chiefly from leaves unless flowers are specified.

[b] Abbreviations: ad. = adults, and l. = larvae.

characteristic symptoms (table 1), and, although not shown by the table, when thrips reared through several generations on such diseased Emilia plants were transferred to pineapple seedlings, the typical yellow-spot symptoms developed. Several collections of these thrips have been determined by Dudley Moulton as the common onion thrips, *Thrips tabaci*, an insect that formerly had not been reported from the pineapple.

These preliminary tests showed that thrips from diseased Emilia were able, in some way, to cause the same disease in Emilia and yellow spot in pineapple. Also, they showed that thrips from certain other plants, even those not showing any recognized symptoms of virosis might do similarly, while thrips from some other plants might not. Contradictory results with different collections from radish flowers from the same field, collected at different times, emphasized the fact that the mere collection of infective thrips from a certain host plant might not indicate the true source of the

TABLE 2.—*Virus transmission by thrips collected from diseased plants of Emilia sagittata from the same intercycle field on different dates*

Insects		Test plants					
		Pineapple			*E. sagittata*		
Collection date	Number per plant	Tested	Infected		Tested	Infected	
			Number	Per cent		Number	Per cent
October 1, 1930[a]	1 adult	10	1	10			
"	5 adults	10	2	20			
"	25 "	10	3	30			
"	5 larvae	10	1	10	10	10	100
(Reared from pupae from Oct. 1 collection)	5 adults	5	5	100	5	4	80
December 12	10 "	38	32	84			
17	10 "	49	39	80			
21	10 "	19	15	79			
28	10 "	27	22	81			
30	10 "	43	35	81			
January 2, 1931	10 "	31	27	87			
5	10 "	33	32	97			

[a] On this date many Emilia plants in this field were healthy and the infected plants were chiefly in early stages of the disease as shown by localization of symptoms on the younger leaves. On the later dates almost all of the larger Emilia plants had long been diseased.

assumed virus, since adult thrips may move from plant to plant and even the larvae may possibly do so where plants of the different species are in close contact. Consequently, it was recognized that infective insects from some of these plants may have acquired the virus while feeding on Emilia or other plants.

DETAILED STUDIES OF VIRUS TRANSMISSION

To determine more fully the rôle of *Thrips tabaci* in the transmission of yellow spot and to provide a sound basis for virus-host studies, a detailed investigation of transmission was undertaken. This involved preliminary work in the development of suitable techniques of rearing, handling, and testing insects. Since only an open-sided greenhouse was available for this work, special attention had to be given to the question of insect cages.

Stock colonies of thrips. Colonies of infective thrips were maintained readily at all times on diseased Emilia plants in the individual plant cages described below.

Noninfective colonies for use in these studies were started from two sources by a method similar to that of Stahl and Carsner (8), as follows:

Adults from infective colonies on Emilia and adults from a field collection from peas, *Pisum sativum* L., were allowed to deposit their eggs in leaves of healthy Emilia and of pea. After 4 days the eggs were subjected to observation under a binocular microscope and, as they hatched, the larvae were transferred immediately, before they fed, to separate seedlings of Emilia and of pea. In this way 13 individual colonies, each of which developed from a single larva, were established.[6] All of these colonies, when tested both on pineapple and on Emilia, have proved consistently noninfective. Only a few were retained, and this work has been done chiefly with one of them.

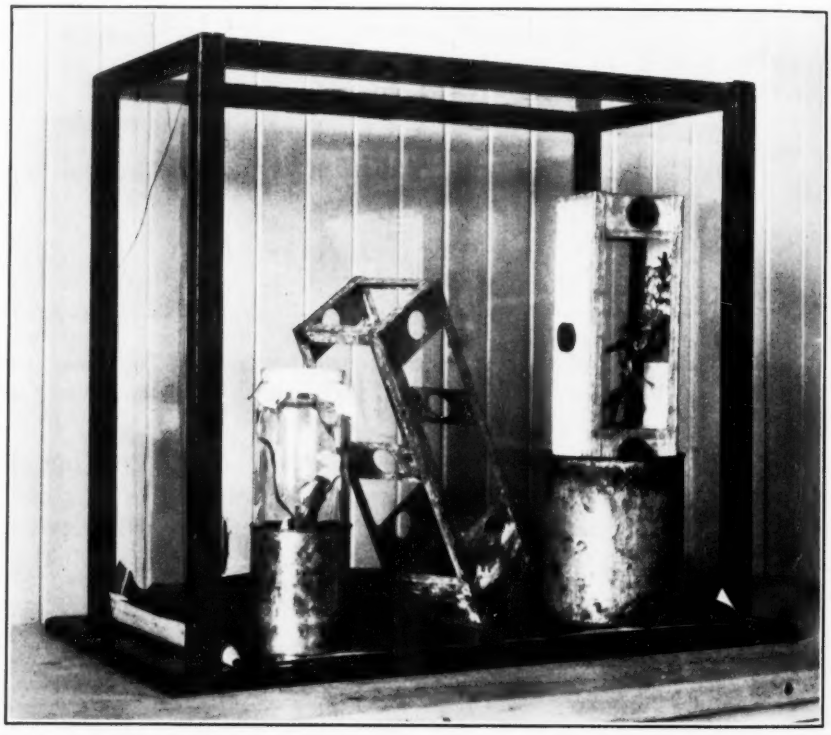

FIG. 6. Transfer chamber, individual plant cage, stock colony cage, and the galvanized-iron frame of the latter, as used in experiments with thrips.

Insect cages. The stock colonies have been maintained in special insect cages constructed, as shown in Figure 6, of galvanized iron, clear sheet pyraline, and a heavy grade of white cotton broadcloth, over pea seedlings

[6] *Thrips tabaci* is parthenogenetic in its reproduction, and all or nearly all of the individuals encountered in such colonies have been females. Thus there has been no difficulty in rearing colonies from single individuals.

grown in gallon canners' tins (No. 10) of soil. Rubber stoppers, size 6, fitted into smooth-cut holes in the metal, made other openings unnecessary. The metal base of the cage was thrust about 2 in. into the soil. The surface of the soil was then sealed around the plant stems by pouring a layer of soft wax (paraffin wax, 5 parts; white petrolatum, 2 parts; and beeswax, 2 parts, by weight). This wax was covered with a layer of air-dry, fumigated soil to provide a suitable place for pupation. Water was applied to the soil outside of the cage and did not wet the dry soil within.

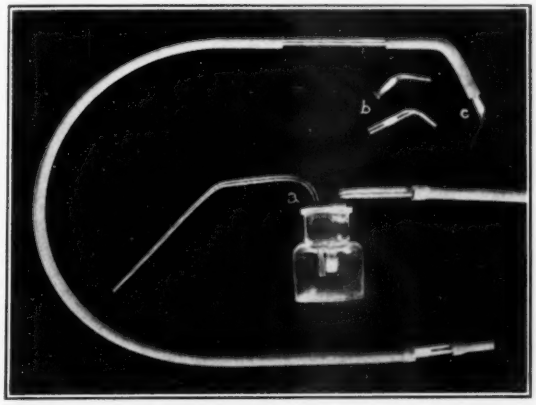

FIG. 7. Apparatus for handling thrips. a. Suction apparatus for collecting thrips into a bottle. b. Small glass pipettes plugged with cotton. c. A similar pipette in a suitable holder of glass and rubber tubing.

Under these conditions thrips reproduce rapidly on peas and monthly transfers to fresh colony cages have been necessary. With a start of 15 adults on 4 plants, it is customary to obtain over 100 adults and many larvae by the time the plants are killed after a month or 6 weeks. For still larger colonies, where less precision was required, large cages of somewhat similar construction to those described above have been used over wooden flats, dispensing with the wax seal.

For individual-plant inoculation chambers, wide-mouth glass jars of 1-qt. capacity were used. The bottoms of these were removed with an electrically heated resistance wire, and the tops were covered with white broadcloth. These jars were pressed into the soil in the quart-size (No. $2\frac{1}{2}$) canners' tins, which were used as plant containers (see Fig. 6).

Handling the insects. Suitable methods of handling thrips were adapted from standard entomological techniques as follows: Suction apparatus of various types was used extensively. For collecting thrips into bottles or vials for various purposes, the apparatus shown in figure 7, a, was very useful when connected with a suction line or with the mouth.

Small pipettes (Fig. 7, b), plugged with cotton were used extensively in transferring adults. The desired number of insects was drawn into the pipette and then the pipette itself was slipped out of the rubber tube and placed in the appropriate insect cage where the insects were allowed to emerge. This proved more satisfactory than forcibly expelling the thrips from the pipette. Larvae and sometimes individual adults were transferred with the moistened tip of a fine brush.

All insect transfers were made in a laboratory room some distance from the greenhouse. The actual transfers were made over black oilcloth in the open-front transfer chamber (Fig. 6), which was thoroughly cleaned between transfers of different lots of thrips.

Plant materials. The pineapple plants used in these tests were grown from seed (chance seed of Cayenne variety) in covered dishes and transplanted into individual plant chambers in time to resume growth before use. The Smooth Cayenne variety is heterozygous and its seedling progeny are highly varied in character. This explains certain variations in the behavior of individual plants.

Emilia sagittata was propagated both from seed and cuttings. Seedlings start slowly and, in the dim light of insect cages, are slender. Stem cuttings of older plants strike root rapidly and provide stronger plants. Propagating materials were handled both in large metal-frame cages over flats and in individual-plant cages. Throughout the greater part of this work soil was subjected to heavy applications of carbon bisulphide, since both Emilia and peas were found to grow very poorly in steam-sterilized soil.

The pineapple plant, even as a young seedling, appears relatively unfavorable for the growth and reproduction of *Thrips tabaci*, and irregular results were obtained unless 5 or more vigorous thrips were applied to each plant. Consequently, *Emilia sagittata*, which is a favorable host plant, has been used extensively. Young larvae, when transferred to it, will mature and reproduce. Symptoms are as characteristic in Emilia when well developed as in pineapple. In the later work, therefore, test insects were applied first to healthy Emilia. If the symptoms that developed were at all uncertain, these thrips or their progeny were later transferred from these Emilia plants to pineapple. Development of the characteristic disease in pineapple was thus the ultimate criterion in these tests.

EXPERIMENTS

The more significant experiments bearing upon virus transmission by thrips are summarized in tables 3 and 4. Insect-free test plants, although not indicated in these tables, were used freely throughout this work, and none of them became infected. Also, though not indicated in the tables,

thrips from test Emilia plants in many of these experiments were transferred to pineapple seedlings to confirm the positive or negative indications.

Experiments with adults. Before the special noninfective colonies of thrips were available, experiments were begun with thrips from other sources. Earlier tests (Table 1) had shown thrips from peas and from Jack bean to be noninfective, so these were used in experiments 1 and 2 of table 3. Adults were fed upon leaves of diseased pineapple and Emilia, with proper checks, and then transferred to test plants. Over 150 adults were exposed to diseased leaves for 1 to 4 days, but none transmitted the virus.

When the pedigreed noninfective colonies, reared as described above, were of sufficient size, thrips from them were used in further experiments (4, 5, 6, and 7 of table 3). Adults were confined for different lengths of time upon excised diseased leaves of Emilia and pineapple, in small wide-mouth bottles covered with broadcloth. After this period of experimental feeding the insects were liberated upon the test plants where, in the case of Emilia at least, they fed freely and reproduced. Even after the 11- and 10-day periods of experiments 4 and 6, respectively, the thrips fed vigorously upon the test plants. The consistent failure of transmission in these tests and in others of a minor nature not tabulated was extremely puzzling.

Experiment 7 of table 3 was a notable exception in that very satisfactory transmission to both Emilia and pineapple was obtained. As far as was recognized at the time, this experiment differed from the others of table 3 only in the somewhat longer period of exposure to diseased leaves, and the difference between the 10 to 11 days of other tests and 14 days of this one did not appear significant because the experimental thrips were allowed to remain on the test plants after transfer. After repeated failure to duplicate these results, it was recognized finally that, in this test, larval progeny of the experimental insects were probably transferred to the test plants.

Experiments with progeny. Experiments that utilized a different method indicated the probable difficulty. Insect-free plants of diseased Emilia, grown vegetatively from 1 plant, were set into separate inoculation chambers and thrips from a noninfective colony were placed upon them to feed. After 4 weeks, when thrips were transferred to test plants, 5 of the 8 colonies proved infective. The duration of exposure to diseased Emilia was such that the progeny had had time to come to maturity. Consequently, it appeared probable that transmission was by the progeny rather than the parent insects.

To test this, experiment 1, A to D, of table 4 was organized more precisely along similar lines. Four mildly blotched, thrips-free cuttings of 1

TABLE 3.—*Experiments with adult thrips showing their failure to become infective*

Experiment number	Insects			Test plants			
	Source	Experimental feeding (adults only)		Applied to each plant[b]	Emilia		Pineapple	
		Exposed to—	Duration		Tested	Infected	Tested	Infected
			(Days)					
1	Peas, open field	0[a]	0	5 ad.	20	0	10	0
	" " "	Healthy pineapple	2	2 to 6 "	12	0		
	" " "	Diseased "	2	2 to 6 "	6	0	—	—
	" " "	" Emilia	3	3 to 5 "	10	0	—	—
2	Jack bean, field (reared on peas)	0	0	1 to 2 "	10	0	—	—
	" " "	Diseased Emilia	1	1 "	5	0	—	—
	" " "	" "	2	1 "	5	0	—	—
	" " "	" "	4	1 to 4 "	10	0	—	—
	" " "	0	0	5 "	—	—	12	0
	" " "	Diseased pineapple	3	5 "	—	—	12	0
3	Stock colony	Healthy "	3	5 "	5	0	9	0
	" "	Diseased "	3	5 "	4	0	6	0
4	" "	Healthy Emilia	3	5 "	5	0	5	0
	" "	" "	11	5 "	5	0	5	0
	" "	Diseased Emilia	3	5 "	5	0	5	0
	" "	" "	5	5 "	5	0	5	0
	" "	" "	7	5 "	5	0	5	0
	" "	" "	9	5 "	5	0	5	0
	" "	" "	11	5 "	3	0	3	0
5	" "	Diseased Emilia, then healthy Emilia	4, 6	5 "	—	—	8	0
6	" "	Diseased pineapple, then healthy Emilia	7, 3	4 to 5 "	3	0	4	0
7	" "	0	0	5 "	5	0	5	0
	" "	Diseased Emilia	14	4 to 5[c] "	10	8	10	6

[a] Transferred directly to the test plants with no experimental feeding.

[b] Where two numbers are listed, the smaller generally represents thrips transferred to Emilia, the larger to pineapple. Adults are represented by ad.

[c] Probably both adults and their larval progeny; no record on this point.

diseased Emilia plant were grown to large size in colony cages and 50 or more adults from noninfective colonies were added to each. After 5 days, adults were removed to test plants. After 19 to 20 days large larvae were removed and tested. These were the progeny of the adults introduced. From all 4 colonies the adults were noninfective. Larvae were tested from only 3 (plant C died early), and from 2 of these colonies they proved highly infective. Note that of 24 Emilia test plants that received larvae from D, 23 became infected.

Experiments 2A, 2B, 3 and 4 of table 4, were of similar nature, comparing adults with their larval progeny reared on 3 other hosts of the yellow-spot virus. Consistently, the adults from noninfective colonies remained noninfective after a period of feeding upon the diseased plant, while their progeny became infective.

Experiments with larvae. Experiments 2C, 5, 6, and 7 of this same table represent a different procedure in that thrips larvae from noninfective colonies on peas were fed in bottles on excised leaves of the infected plants. In each instance, larvae became infective after even 1 day's exposure to the source of virus. In experiments 5 and 7 of table 4, special care was taken to eliminate other variables. The adults used were from a young colony and most, if not all, were young adults. The larvae were collected from the same individual pea plants as were the adults. The several leaves used as the source of virus were each split in half, 1 half going to the larvae, the other half to the adults. The results of these experiments, therefore, in addition to the ample evidence of the others of tables 3 and 4, show conclusively that larvae from noninfective colonies acquire the virus readily by feeding on infected plants of different species but that adults, treated similarly, do not.[7]

Retention of virus through pupation. At the same time it is plain that thrips that have acquired the virus as larvae retain their infective capacity as adults. This is shown by experiment 5 of table 4, in which some of the larvae were allowed to pupate and later, after maturing, were tested as adults and found infective. The same situation is revealed by the experiment with field-collected insects reported in table 2.

Transmission by larvae. Since, in studies reported above, the thrips were allowed to remain indefinitely upon the test plants where, in the case of Emilia at least, larvae developed into adults, these experiments did not show whether larvae, as such, actually transmit the virus. The general association of oviposition marks with initial spots in the field suggests that

[7] Since obtaining these results the writer has learned by conversation with Dr. Geoffrey Samuel, while he was in Honolulu, that he has come to similar conclusions with respect to the transmission of the tomato spotted-wilt virus by *Frankliniella insularis* in Australia.

[b] Where two numbers are listed, the smaller generally represents thrips transferred to Emilia, the larger to pineapple. Adults are represented by ad.

[c] Probably both adults and their larval progeny; no record on this point.

TABLE 4.—*Experiments comparing adults and larvae in their ability to become infective*

Experiment number		Insects				Test plants			
		Experimental feeding			Applied to each plant[a]	Emilia		Pineapple	
		Stage	Source of virus	Duration		Tested	Infected	Tested	Infected
				(Days)					
1	A	Adult	0	0	5 ad.	5	0	5	0
		"	Emilia	5	2 to 5 "	6	0	3	0
		Egg[b]	"	20	3 l.	10	8	...	...
	B	Adult	0	0	2 to 5 ad.	5	0	5	0
		"	Emilia	5	2 to 5 "	5	0	3	0
		"	"	20	5 "	...	...	8	0
		Egg[b]	"	20[c]	3 l.	4	0	...	...
	C	Adult	0	0	3 to 5 ad.	5	0	5	0
		"	Emilia	5	2 to 5 "	5	0	4	0
	D	"	0	0	3 to 5 ad.	5	0	5	0
		Egg[b]	Emilia	19[c]	3 l.	24	23	...	...
		"	"	19	5 to 8 l.	...	...	3	3
2	A	Adult	*Richardsonia scabra*	5	3 ad.	4	0	...	...
		Egg[b]	" "	14[c]	4 l.	5	5	...	...
	B	Adult	" "	5	3 ad.	4	0	...	...
		Egg[b]	" "	14	4 l.	5	5	...	...
	C	Larva	" "	2	4 l.	5	5	...	...
3		Adult	Ageratum	2	5 ad.	4	0	4	0
		Egg[b]	"	18[c]	2 l.	2	2	...	...
4		Adult	Stachys	5	3 ad.	4	0	...	...
		"	"	14	3 "	5	0	...	...

TABLE 4.—(*Continued*)

Experiment number	Insects				Test plants			
	Experimental feeding			Applied to each plant[a]	Emilia		Pineapple	
	Stage	Source of virus	Duration		Tested	Infected	Tested	Infected
4 (cont.)	Larva	"	2	4 l.	3	3	...	...
5	Adult	0	0	5 ad.	5	0	5	0
	"	Emilia	1	3 to 10 "	3	0	9	0
	"	"	4	3 to 5 "	5	0	3	0
	Larva	"	1	3 to 5 l.	4	4	1	0
	"	"	17[d]	1 or 2 ad.	5	5	...	...
6	"	"	1	4 l.	11	10	...	...
	"	"	1	4 l.	8	7	...	...
	"	"	1	4 l.	6	6	...	...
7 A	Adult	Pineapple	2	3 ad.	5	0	...	...
	Larva	"	2	3 p.	5	1	...	...
B	Adult	"	2	3 ad.	5	0	...	...
	"	"	2	2 to 10 "	...	...	5	0
	Larva	"	2	3 p.	5	4	...	...

[a] Where 2 numbers are listed, the smaller generally represents the thrips transferred to Emilia, and the larger to pineapples. Adults are represented by ad., larvae by l, and pupae by p.

[b] Thrips hatched from eggs deposited in the diseased plant, source of virus, and reared thereon.

[c] This represents the period from liberation of parent adults on the virus source to the transfer of larval progeny to test plants.

[d] These insects fed on the virus source until they pupated. The matured adults fed only upon healthy Emilia prior to being tested.

the pineapple, at least, is inoculated generally by adults. Special tests were made, therefore, with larvae confined in cotton-plugged glass capsules upon individual leaves of Emilia and later recovered from the plants. Four plants, thus inoculated, developed the disease, proving that larvae do transmit the virus.

Incubation period in the plant. During the experiments outlined above and others not reported in detail, observations of test plants have been made, generally, at intervals of 3 days. In some tests they have been more frequent at the outset to determine the minimum incubation period with accuracy. Data collected in this way (Table 5, line C) indicate a minimum

TABLE 5.—*Incubation periods in pineapple, Emilia, and Thrips tabaci. An incubation period in the insect is indicated by comparison of apparent incubation periods in Emilia plants inoculated by insects of different histories*

Thrips used in tests	Test plants	Apparent incubation period		
		Minimum	Maximum	Mean
		Days	Days	Days
A. Chiefly adults from diseased plants	161 pineapple seedlings	7	27+	12.2
B. Larvae fed one day on infected plant and left to mature upon the test plant	48 *Emilia sagittata*	18	35	24.9
C. Large larvae and adults reared on infected plants	82 " "	8	27	14.6
Indicated incubation period of virus in thrips (B minus C)		10	8	10.3

incubation period in Emilia of 8 or 9 days and a mean period of 14.6 days calculated on the basis of 82 plants. The approximate maximum of 27 days is not very significant, owing to less frequent observation at the end of an experiment, and, similarly, the mean of 14.6 days is probably too great as a consequence of not making daily observations throughout. Part of the variation in apparent incubation period is a result of different rates of growth, since symptoms in this plant depend for their expression on the development of new leaves from the growing point.

The incubation period in the pineapple is less readily determined with accuracy, because the initial symptoms develop at the point of feeding and because the feeding of even noninfective thrips may cause a conspicuous local yellowing. Here, again, the rate of growth enters as a factor, because

thrips inoculate the pineapple leaf near its base, where other leaves hide the symptoms until the leaf elongates from below, lifting the initial spot to view. The observed incubation periods in 161 plants are shown in table 5, line A. Seven days was the minimum and 12.2 days the mean. Again, as with Emilia, this mean is perhaps slightly high because observations were not made daily. More limited observations on young plants grown from crowns in the field indicate the incubation period to be about 2 weeks.

Incubation period in the insect. In the course of these studies it was clearly apparent that when Emilia plants were inoculated by thrips reared on diseased plants, symptoms developed in fewer days than when inoculation was by thrips that had fed as larvae for only 1 day on a diseased plant prior to being placed on the test plant. Table 5 compares the minimum, maxi-

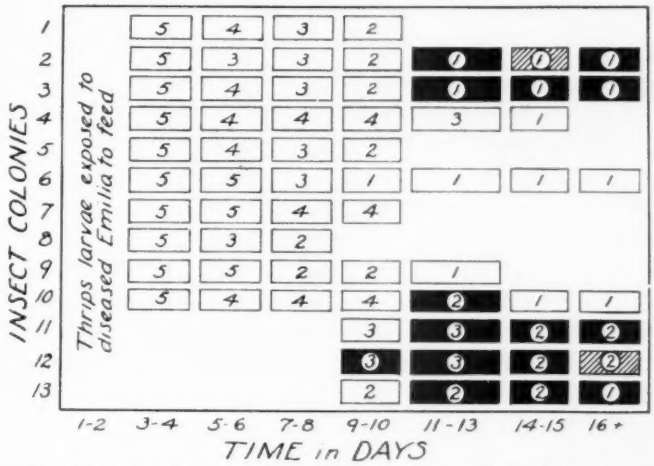

FIG. 8. Incubation period of virus in *Thrips tabaci*. Rectangles represent individual test plants on which thrips fed during the days indicated below each column. The number within each represents the thrips placed upon the plant. Black rectangles represent infected plants, and shaded rectangles plants that died. The declining numbers of insects in each colony represent loss of thrips chiefly by death. Three of the infected plants were inoculated by larvae, the others by adults.

mum, and mean periods, in days, between adding the insects and observing symptoms in 82 and 48 plants inoculated in these two ways. Assuming the actual period of incubation in the test plants to be the same in both cases, the difference between the apparent periods must be a measure of the length of incubation period in the insect. These results suggest, on this basis, an incubation period of the virus in thrips of about 10 days. Kunkel (3), working with aster yellows, found that the incubation period in the plant may be shorter when inoculation is by an insect that has carried the virus a long time than when by an insect that has just become infective. Possibly, therefore, the incubation period in thrips may be somewhat less than

10 days. The use of different numbers of insects in these tests did not invalidate results, since the shortest incubation period observed, 8 days, and several other short periods were observed following inoculation by a single adult.

A more direct test for incubation period in thrips is reported in figure 8. Thrips larvae of mixed ages were transferred from a noninfective colony on peas to an excised leaf of diseased Emilia. On the 3rd day, 5 of these larvae were confined in single-leaf cages upon each of 10 healthy Emilia plants. After 2 days they were removed from these plants and caged upon others, and so on to the 16th day, when the insects that survived were liberated on Emilia plants for the rest of their lives. On the 9th day, 3 additional colonies were started with adults reared on excised leaves of healthy Emilia from larvae that had fed on diseased Emilia the same as colonies 1 to 10. Some colonies died out entirely, some insects died in all colonies, and 2 test plants died without exhibiting symptoms. It will be seen from the figure that 1 of the plants, fed on from the 9th to the 10th day, became infected. The first transmission by the other colonies was to plants fed on from the 11th to 13th day.

The results of this test indicate, in confirmation of the above evidence, that thrips are noninfective until approximately 10 days after they first feed, as larvae, upon an infected plant. No more precise determination has been attempted. It must be recognized that this period is close to the maximum duration of the larval stages under favorable conditions and that the intervention of the pupal stage may be a factor in lengthening the apparent period of incubation in the insect. Still, in this test, 3 plants were inoculated by either large larvae or prepupae. Clearly, no close interpretations are permissible until more detailed studies are carried out with closer attention to the insect itself.

DISCUSSION

Among the insects that transmit plant viruses the Thysanoptera (thrips) have received consideration only recently. Several workers have suggested that species of thrips might be vectors of different viruses, but the present case appears to be only the second one supported by adequate experimentation. The report on spotted wilt of tomato by Samuel, Bald, and Pittman (7) indicates several points of similarity between the 2 viruses and their relation to their vectors.

The method of finding the vector appears particularly interesting in that *Thrips tabaci* was not formerly known ever to feed upon the pineapple.[8] Even now it appears to inoculate the pineapple only incidentally

[8] Although a prelminary report of the Australian work with thrips had already been published by Pittman (6), this was unknown to the writer until he had found, by independent means, that *Thrips tabaci* is the vector of yellow spot.

when it has left its preferred hosts. On some young pineapple seedlings in the greenhouse a new generation will develop, but, even here, where conditions favor rapid reproduction on suitable host plants, a colony of thrips usually disappears within 10 days or less. Eggs that are laid in the leaves hatch and the young larvae feed for a time, but it seems probable that this species of thrips seldom comes to maturity on young pineapple plants in the field.

Clearly, the pineapple is not a suitable colony plant for rearing thrips. Furthermore, since pineapples are quickly killed by the disease, they are not a satisfactory source of the virus for experimental use. It would have been very difficult to carry on these studies of transmission were it not for the early recognition of a more suitable host of both virus and thrips in *Emilia sagittata.*

As indicated in table 4 and as will be discussed more fully in a later paper, several other hosts of the virus have since been found,[9] but this species of Emilia still appears to be of the greatest significance in the occurrence of pineapple yellow spot. Not only is it a favored host of the insect and highly susceptible to infection, but it lives long in the diseased condition and thus may maintain a colony of infective thrips for several months. Moreover, it is one of the most common weeds in pineapple fields, in intercycle fields, and in nongrassy waste lands in those areas where yellow spot is prevalent. Almost pure stands of it may develop in such areas, producing a great population of viruliferous insects.

Were it not for this and other hosts of the virus, the pineapple yellow spot might be self-exterminating, since the pineapple plant infected with yellow spot soon dies. Consequently, transmission of the virus in diseased planting material within an infested region is not of serious importance and roguing is not needed as a control measure. Dead and dying plants are removed from fields simply to make space for replants. It is the movement of infective thrips from other hosts of the virus that accounts for the appearance of this disease in the pineapple.

From the standpoint of dissemination of this virus it is significant that noninfective adult thrips, in migration, may feed upon diseased host plants and then move off to other plants without transmitting the disease. It is the progeny of these adults, grown to maturity on infected plants, that will transmit the disease when they in turn move to other plants. As shown by table 2, noninfective adults may be collected from recently infected host plants.

There is no reason to think that this is the only species of thrips that may transmit this virus. Other species have not been tested. Since, how-

[9] The incomplete list of hosts includes members of the following families: Bromeliaceae, Liliaceae, Caryophyllaceae, Leguminosae, Labiatae, Solanaceae, Rubiaceae, and Compositae.

ever, *Thrips tabaci* is the most commonly observed species on the known hosts of the virus it seems clear that it is the chief vector. In the case of spotted wilt of tomato (7), the known vector, *Frankliniella insularis*, is absent from part of the range of the disease, indicating that at least a second vector must be involved. That insects other than species of thrips do not transmit to the pineapple often, if at all, is indicated by the close association of thrips ovipunctures with initial spots.

Control of yellow spot in pineapple thus resolves itself into the problems of reducing the reservoir of the virus in wild hosts, controlling the insect, and breeding resistant varieties of pineapple. Of these, thrips control offers most promise of early results. This is being studied by the Entomologist. Marked differences in the behavior of the heterozygous pineapple seedlings used in these studies indicate that significant resistance may be found.

The detailed studies of transmission are of peculiar interest in that they demonstrate a specific relationship between virus and insect in the case of a disease that appears, on the basis of symptoms, to be more closely related to the mosaic than to the yellows type of disease. As has been suggested by Kunkel (3, 4) in the transmission of aster yellows by *Cicadula sexnotata*, the presence of an incubation period of the virus in the insect seems to support the view that the virus is in the nature of a living entity rather than merely a chemical. The incubation period in *Thrips tabaci* is longer than seems to be recorded for any virus except that of aster yellows. Its close approach to the total duration of the larval stages, together with the fact that larvae only are able to acquire the virus, complicates the present case and indicates the need for a further study of the larval stages separately.

It is only possible to speculate upon the mechanism that prevents adult thrips from becoming infective, even though thrips that have earlier acquired the virus as larvae may transmit freely in their adult stage. Clearly, the feeding of infective adults introduces the virus into the plant in a manner suitable for infection. Moreover, adults doubtless ingest the virus during their feeding on diseased plants. The difference appears to lie in some factor that prevents the virus from moving out of the digestive tract and into organs from which it may be ejected during feeding. Some fundamental change in the physiology of the insect seems indicated. Possibly, this is a change in digestion such that, in adults, the virus is promptly destroyed, whereas in the larvae it is not harmed but is able to migrate from the digestive tract to other organs. Or the difference may result from a change in the insect that prevents migration of the virus to the salivary glands or other parts from which it may be ejected.

SUMMARY

This paper presents in more detail the work summarized in a brief former note and reports further studies dealing with transmission of the virus of pineapple yellow spot by *Thrips tabaci*.

The identity of the vector, an insect not formerly reported from the pineapple, was inferred from a study of its oviposition marks associated with the initial stages of this disease.

A weed host of the virus, *Emilia sagittata*, which also is a favored host of *T. tabaci*, was recognized because of similarities of symptoms and of space and time distribution.

Thrips reared on this plant were found to transmit the virus to both Emilia and pineapple.

For detailed studies of transmission, noninfective colonies of thrips were reared from single larvae.

Methods of rearing, handling, and testing thrips used in these studies are described in full. Since the pineapple is not a suitable colony plant, Emilia and garden pea have been used extensively.

These studies have shown the following:

Larvae from a noninfective colony become infective after feeding upon a diseased plant, but adults treated similarly do not. The virus survives pupation, and insects that feed on a source of virus while larvae may be infective as adults.

There is a period of approximately 10 days after first feeding on a diseased plant before thrips transmit infection.

A single insect, larva or adult, may transmit the virus to Emilia.

The minimum incubation period in *E. sagittata* is about 8 days and the mean about 15 days. In young pineapple plants the minimum is 7 days and the mean about 12 days.

Thrips tabaci transmits the virus to and recovers it from several other plants in addition to pineapple and Emilia. These are to be considered in a separate paper together with symptom studies.

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THE DEVELOPMENT OF THE ROOT-KNOT NEMATODE IN RELATION TO ROOT TISSUES OF PINEAPPLE AND COWPEA[1]

G. H. Godfrey and Juliette Oliveira

INTRODUCTION

There is need for more detailed information than has been available on the life history of the root-knot nematode, *Heterodera radicicola* (Greef) Müller, in relation to root tissues. This need is manifested by the lack of agreement between early investigators with regard to certain life-history details and by the lack of definite information on other points. These studies have clarified to some degree the details of time and manner of penetration of the larvae, length and characteristics of the motility period within the roots, orientation within the root tissues, relation of developmental stages to roots, and, finally, the time and duration of egg development. This graphic presentation of the main developmental stages of the nematode shows clearly the nature of the things that are happening within the root during the development of the nematode gall.

LITERATURE REVIEW

The first mention of root knot in the scientific literature was by Berkeley (2) in 1855. Though the causal organism was not described in any detail, the illustrations, both of the type of the disease and of the organism related to it, leave no doubt as to its identity. Since then, scores of papers have been written on the subject. The literature cited by Bessey (3) in 1911 is nearly complete in the way of reference to the early papers. We shall confine our references to those papers dealing strictly with our subject.

Atkinson (1) deals mainly with complex nematode galls, as he found them in the fields, and does not attempt a description of the relatively simple relationships existing in a primary infestation of a root. Frank (8, pp. 19–24) and Stone and Smith (20) add little to this, emphasizing mainly the complexities of the tissue relationships in the more advanced galls. Molliard (14) presents clearly a description with illustrations of typical multinucleate giant cells produced in the root tissues in the vicinity of the head end of the nematode. Tischler (21) describes in greater detail these giant cells with the histological details of mitotic and amitotic division of their nuclei. Bessey (3, p. 30) describes very briefly primary penetration

[1] Technical paper No. 27 of the Experiment Station of the Association of Hawaiian Pineapple Canners, University of Hawaii.

and arrangement of the nematodes in the tissues, stating that they may be either within the central cylinder or entirely in the cortex. In either case "the anterior end . . . is usually in close connection with the cells surrounding the conductive tissue." He mentions the fixation of the nematodes in the tissues and gives details regarding further development of the organisms. He refers to the host reaction as "hypertrophy of the tissue," the parenchyma cells becoming "abnormally large and multinucleate," referring to Tischler (21) as his authority. Occasional other investigators have made mention of this giant-cell formation without adding materially to the understanding of it. Byars (4) describes inoculation under pure culture conditions and describes initial penetration and initial host reactions from the point of view of external appearance. None of these writers presents accurately the picture of the position-and-time relationship of *Heterodera radicicola* to plant tissue, to gall development, and to reproduction; none differentiates clearly between the simpler primary reaction and the more complex phenomena that develop from secondary infestation of the tissues. Sengbusch (19), in studies on *H. schachtii* Schmidt, includes several photomicrographs of nematodes in process of development within root tissues and shows diagramatically their position in the roots in a case of general infection.

Many investigators give figures in round numbers as to length of life history, numbers of eggs produced, time required for hatching, etc. Reference will be made to specific papers in our discussion of these phases of the study.

METHODS

The general plan of procedure was to inoculate simultaneously a large number of root tips of two host plants, pineapple, *Ananas sativus* Schult., and cowpea, *Vigna sinensis* Endl., with Heterodera larvae, to excise the roots at intervals from time of the first penetration up to maturity of the nematode, and to preserve them for examination. As the first step, several pineapple plants were started in sterilized soil in the smaller root-observation boxes, developed at this station and described by Dr. Dean (6). Within 1 to 2 weeks after planting, roots were well started. Those in appropriate condition for inoculation are illustrated in an earlier paper by Godfrey (11, Fig. 5). Inoculation was made by applying at each root tip approximately 500 larvae produced by incubation of egg masses according to the method described by Godfrey (12), from cowpea roots such as are shown in that paper (Fig. 2).

With cowpeas the procedure was practically the same, preliminary experiments indicating the necessity for minor variations. The cowpeas were likewise planted in root-observation boxes. In 3 or 4 days root develop-

ment was sufficient for inoculation. Time of planting was adjusted so that roots of cowpeas and pineapples would be at a suitable stage for inoculation at the same time. For the entire period of observations inoculations of cowpeas and pineapple were made on the same date. Both lots of plants were therefore subjected to the same temperature range through the period of observations. Water applications were not weighed, but, in general, optima moisture conditions were maintained. Previous results by Godfrey (10) have shown that through the range of soil moistures favorable to plant growth, little, if any, difference in susceptibility obtains.

With both pineapple and cowpea plants 3 or 4 representative inoculated roots were cut at each of the following intervals after inoculation: 6, 12, 18, and 24 hours, and thereafter at day intervals. In every case they were gently washed, until free from adhering soil particles, with the aid of a camel's-hair brush. Killing was in warm (55° C.) Flemming's solution. The resulting instant killing of the nematodes was desirable in that it prevented them from going into unnatural contortions. The osmic acid of the Flemming's solution stained the nematodes black. By removing the roots from the fixative at the right time the root tissues were not overstained. The usual period was between 2 and 4 hours. If, occasionally, too great staining of roots occurred, a few minutes' exposure to strong hydrogen peroxide solution bleached them without reducing too greatly the color in the nematodes. The roots were then washed in running water over night, then dehydrated in graduated series of alcohols according to the usual methods, and cleared in clove oil. The resulting preparations showed to good advantage the growth stages of the nematodes and their relation to the root tissues. As a rule this material lends itself with splendid advantage to photographic reproduction. In many cases the whole root could be used for this. With thicker or more deeply stained roots it was sometimes advisable to slice off the upper and lower portions of the root in longitudinal parallel planes in order to show the stained nematodes to better advantage. Photographs were made with an 8 x 10 Eastman View camera with a special lens board adapted for a 48 mm. microtessar as the only lens. This was mounted on a Folmer and Schwing upright camera stand and had the additional provision of front and rear clamps to prevent vibration of the camera. In the illustrations herein presented magnification is 16 diameters throughout, except in cases specifically stated to be otherwise.

Permanent microscope slide mounts of the choicer materials chosen for photographing were readily made by mounting the materials directly from clove oil into relatively soft balsam and covering with cover glasses. As the balsam gradually thickened more was added at the edge until finally infiltration was complete. After several months the permanent sides still show the sharply delineated nondistorted nematodes in their natural position in the root tissues.

OBSERVATIONS ON PINEAPPLE

It should be noted that these observations apply only to the particular conditions of this experiment, which was conducted in the summer time. Unfortunately, temperature-control apparatus was lacking and our results cannot be stated in absolute terms, *i.e.*, in terms of stage of development of nematodes at a certain age and temperature.

A detailed chronological account of observations on pineapple roots is first presented. Following this, observations on cowpea roots are recorded with special emphasis upon the differences that were observable between the two plants.

In pineapple the first penetration of nematodes occurred some time prior to 6 hours after inoculation. The 6-hour observation showed that a few nematodes had already penetrated into the interior of the root tips. Between 6 and 12 hours heavy additional penetration occurred, as shown by figure 1, A to D, at 12 hours. It appears at this stage that mass action is

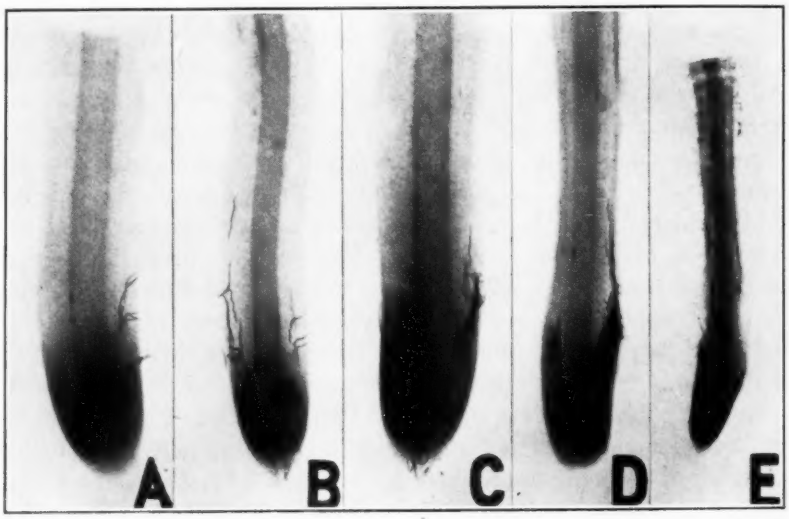

FIG. 1. Early stages of infection of pineapple roots with *Heterodera radicicola*. A to D, 12 hours after inoculation. E, 18 hours after inoculation. The roots are entire (not sections), killed in Flemming's solution, and cleared in clove oil. In A to D note larvae in the act of entering the roots. B and C show larvae entering through the root cap; C and D show evidence of mass activity on the part of the nematodes; E shows heavy infection of a small root. Magnification about 16 diameters.

contributory to penetration, for large numbers of larvae frequently penetrate the root on one side and at or near the same point. Some of the material showed a large number of larvae entering at one side and half in and half out of the root (Fig. 1, A, B, and C). Penetration takes place

mostly on the side of the root just back of the root tip but distinctly in the meristematic region. Occasional early infections show larvae actually at the tip of the root, in the act of penetrating the root cap (Fig. 1, B and C). The 18- and 24-hour material (Figs. 1, E, and 2, A) show continued root penetration.

At 48 hours or even earlier (Fig. 2, B) a semblance of root enlargement occurs. There is evidence that the abundance of infestation in the meristematic region results in retardation of growth of root meristem, since forward growth of the roots has been checked. Large numbers of cases have been observed in which heavily inoculated roots advanced scarcely a quarter of an inch in the first 2 days after inoculation, whereas noninoculated con-

FIG. 2. Pineapple roots. A. 24 hours after inoculation. B. 48 hours after inoculation. A shows migration of the earliest invaders. Root B was sectioned into three parts to show more clearly the abundance of larvae in the root tip region; right, upper portion, middle, central portion, and left, lower portion of the root. Magnification of this and succeeding figures 16 diameters unless otherwise indicated.

trol roots a few inches away had grown 2 inches or more. This seeming enlargement cannot be true gall formation, however, for the larvae are not yet in the position in the roots that is necessary to the first development of true gall tissue. This will be explained in another paper on the histology of the early stages of gall formation. Even at 48 hours, in pineapple root (Fig. 2, B), the apparent swelling is due to retardation of forward growth of the root and abnormally directed growth of the weakened meristematic tissues rather than to true gall development.

These early stages (Fig. 2) all show marked concentration of nematodes in the tip region. There is evidence, however, of the beginning of directed

migration, for larvae can be seen in the cortex parallel with the stele, with their head ends approaching its periphery. In the meanwhile, maturation of tissues goes forward in the root tip and, with it, continued systematic orientation of the larvae. They advance through the cortical tissues and by the 4th day orient themselves typically (Fig. 3, A) with their head ends buried in the region of the clearly differentiated pericycle of the stele and endodermis of the cortex with their bodies extending diagonally outward and downward in the cortex. When once established in this position they begin to feed and immediately to grow. From this time on until maturity, with the male, and permanently, with the female, the position is the same.

FIG. 3. A. Pineapple root 4 days after inoculation, showing orientation of the nematodes and early stages of gall formation. B. Another root 7 days after inoculation, showing the systematic orientation of the nematodes with their head ends embedded in the periphery of the stele.

Larvae were in no case seen thus permanently established *within* the central cylinder, as mentioned by Bessey (3, p. 30), and the writers doubt the occurrence of such arrangement in primary infections. No further migration and, in fact, very little movement of any kind occurs. An immediate host reaction takes place in the form of development of multinucleate giant cells in the immediate vicinity of the phloem vessels, as described by Molliard (14) and Tischler (21). Figure 3, B, at 7 days, shows to excellent advantage the orientation of the nematodes in the cortical tissues and suggests the early stages of developments responsible for formation of the galls.

Here some growth in the form of thickening rather than lengthening of the larvae has already occurred.

From this time on, for several days, growth of the nematode, in the pineapple roots, is rather slow and somewhat irregular. The nematode becomes typically fusiform, then as a result of a period of more rapid development, flask-shape, and finally spherical.

Heavy infestation of a pineapple root as a rule results in complete cessation of forward growth. Occasionally, however, after a period during which forward growth has obviously been checked, it is renewed by a re-

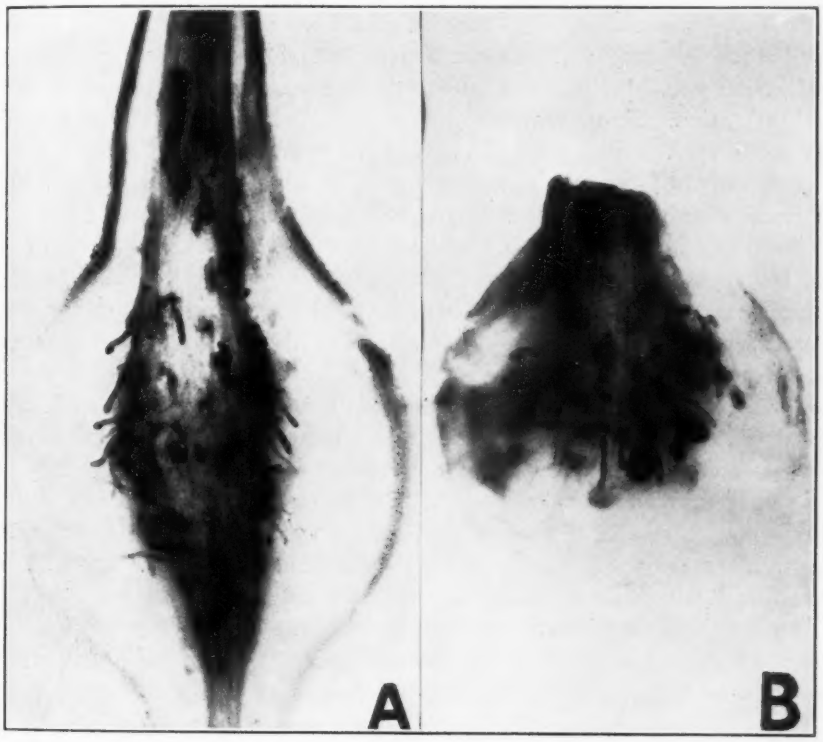

FIG. 4. A. Pineapple root 24 days after inoculation with a nonterminal gall, showing nematodes in the fusiform condition typically oriented in the root tissues. Note darkened regions in the stele where giant cell formation is taking place. B. Pineapple terminal gall 27 days after inoculation, showing great increase in size of some of the female nematodes. Sections 1.5 or 2 mm. thick.

sumption of activity of the meristematic tissues. The result is a normal-appearing root tip growing out of the end of the old gall. This may continue to grow indefinitely as a normal root or it may be checked by another nematode infestation and produce another gall. Continued repetition ac-

counts for the beaded effect in roots, when galled regions alternate with normal root growth. Such a nonterminal gall is shown in figure 4, A, which is an illustration of a root removed 24 days after inoculation. Here are to be noted fusiform nematodes of fairly uniform size, arranged as described, in the cortex with their heads in the periphery of the stele. Attention is called to the region of development of the giant cells directly at the head of the nematode. The effect of the parasites in bringing about a very great enlargement of the central cylinder of the root shows strikingly in this photograph. Note, too, that the cortex is considerably thicker in this region of heavy infestation than in the normal part of the root. This latter appears to be a secondary effect, since there is no evidence of abnormal cell development in the cortex. It is the combined effect of the thickening of the central cylinder and the unusual thickening of the cortex that brings about the gall formation. Examination of the terminal gall shown in figure 4, B, discloses the very rapid increase in size of the female nematode, which clearly occurs sometime between the 24th and 27th days after inoculation. This increase in size is shown to better advantage in figure 5, A, at 30 days, in which many nematodes have become flask-shape. The discoloration showing the region in the central cylinder in which giant cell formation is developing is also well shown in this picture. Figure 5, B, at 35 days, shows a number of fully developed females. The illustrations of development from the 24th to the 35th days show striking irregularities, which would seem to indicate that stage of development is not a function of time, alone, but probably of nutrition, as well. There is no question from examination of these pictures but that some of the females will mature and produce eggs very much in advance of others. It is to be regretted that none of the pictures shows males in the act of undergoing the final molt or of migration through the tissues. It is desirable to know the exact stage at which this takes place. This detail remains for further study.

In the series under observation, not until the 37th day (Fig. 5, C) was clear-cut egg-mass development discernible. This figure illustrates to excellent advantage the extruded gelatinous matrix in which the eggs are found. In two or three of the individuals shown in this picture the egg mass has already attained considerable size. It is to be noted that this egg mass is retained entirely within the cortex, that is to say, it in no place protrudes to the outside of the root as shown in the cowpea material, which was used for source of inoculation. That this has an important bearing on propagation of nematodes in the field will be pointed out in connection with the discussion of cowpea roots later in this paper.

When examining fresh materials of nematode galls it is virtually impossible to obtain a clear idea either of the number of nematodes contained therein or of stages of development of those nematodes. Prepared mate-

rials, such as were used for the illustrations shown here, have made such information readily available. Moreover, they should add considerably to the understanding of the processes of gall development.

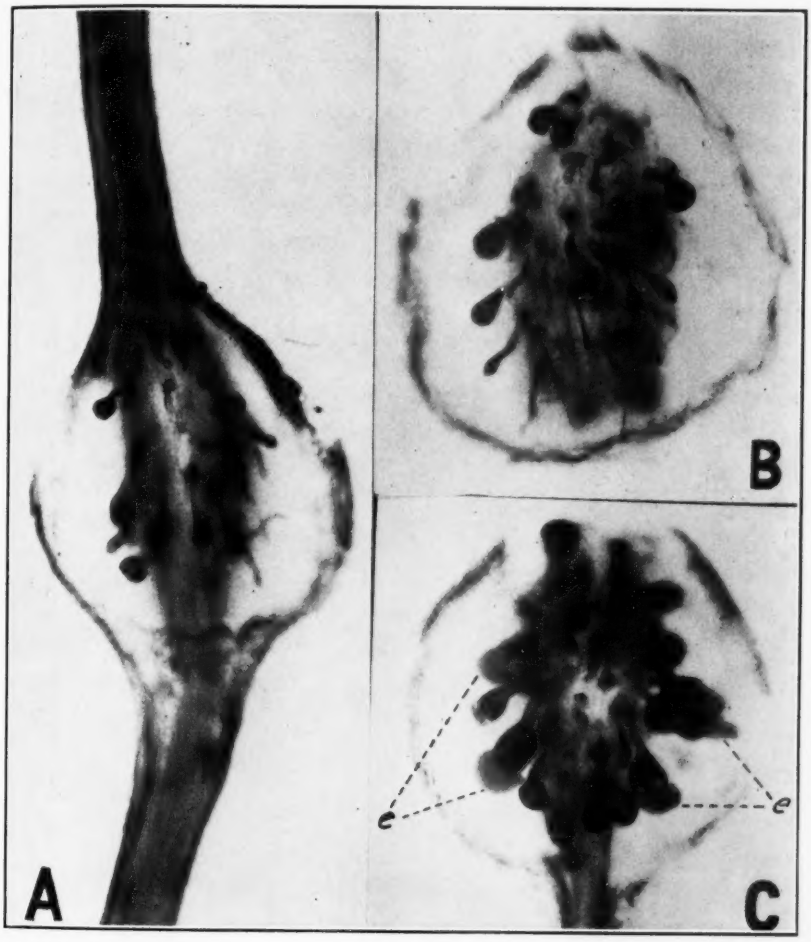

FIG. 5. A. Nonterminal pineapple gall 30 days after inoculation, showing flask-shape females. B. Terminal pineapple root gall 35 days after inoculation, showing fully developed adult female nematodes and many others not fully developed. C. Pineapple nonterminal gall, showing abundant adult nematodes with extruded egg masses (e). Regions of giant-cell formation are clearly shown here. Note also the abnormal thickness of the cortex.

OBSERVATIONS ON COWPEAS

As stated before, procedure with cowpea roots was essentially identical to that with pineapple roots. Because of the much more rapid root growth

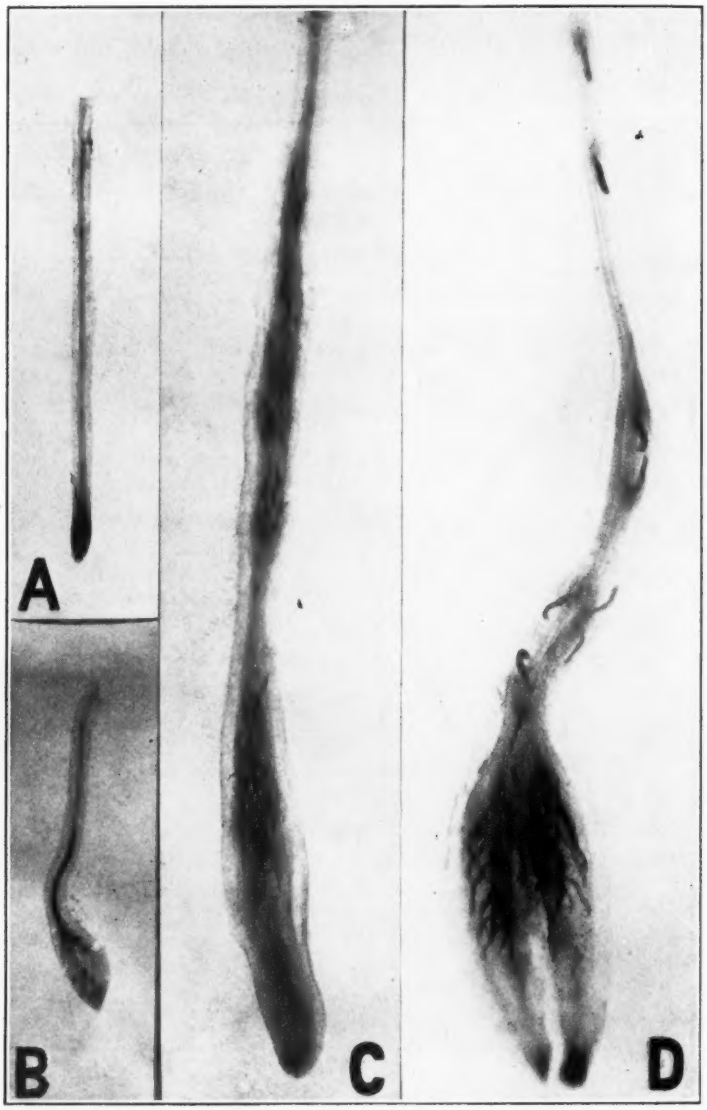

FIG. 6. Cowpea roots. A. 6 hours after inoculation. B. 48 hours after inoculation. Note that terminal swelling has already occurred. C. 4 days after inoculation, showing permanent orientation and beginning of growth of nematodes and distinct gall-tissue development. It is evident from the appearance of the root tip that it was still in active growth when it was cut from the plant. Due to this continuing growth, the nematodes are distributed over a greater length of the root than in pineapple roots of the same age. D. 7 days after inoculation, showing definite orientation of the nematodes in the tissues and distinct increase in size.

and branching in cowpea it was difficult to preserve the identity of originally inoculated root tips. In the later daily selection of more advanced stages of gall development the oldest appearing galls were selected in every case as the representative of the particular age desired. With greater abundance of tender cowpea roots available for infection, as well as more rapid root growth, it appears that there was greater wandering of the nematodes through the soil and, consequently, less concentration in any given root tip. The point of inoculation in the box was carefully marked, however, and it is believed that a fairly reliable range of root ages was selected.

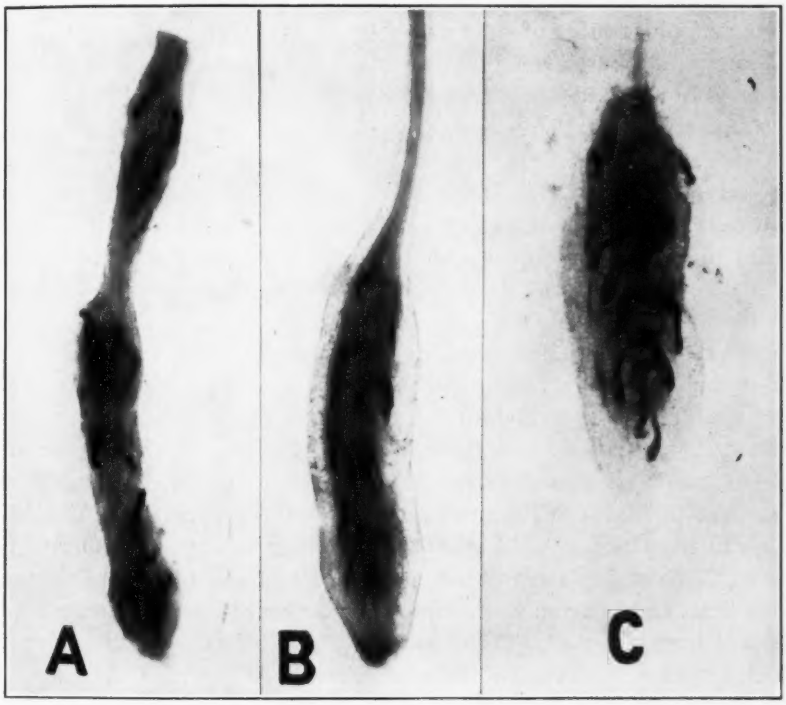

FIG. 7. A. Cowpea root 9 days after inoculation, showing nematodes in the fusiform stage. B. Infection may have been delayed here, for the nematodes do not appear to be so large as in A. This is a typical cowpea terminal gall. It shows enlargement of the stele to be primarily responsible for the root enlargement. C. Cowpea root 13 days after inoculation.

The early stages of penetration of nematodes in cowpea roots appear to be nearly identical with the same stages in pineapple. The first sign of gall development was perhaps manifest slightly earlier in cowpea than in the pineapple root. The cowpea root manifested the capacity to keep on growing, however, to a much greater extent than the pineapple. The result

was, apparently, the distribution of the nematodes within the tissues over a greater length of the root. This was due rather to continued growth of the root than to greater migration of the nematodes through the cowpea tissues. Figure 6, A and B, shows 6-hour and 48-hour stages, respectively, in a cowpea root. Figure 6, C, shows a cowpea root 4 days after inoculation and illustrates the distribution of the nematode through the length of the root. The appearance of the root tip indicates that it was still growing at the time the root was cut. Had the supply of larvae become exhausted in the vicinity of the root tip, the chances are that the root would have continued to grow and that gall formation would have appeared only in the particular region of the root shown in this picture, the root tip itself not being swollen. Already at this stage some of the nematodes had become fixed in the tissues and some growth had taken place. This, of course, means that migration has ceased, except, perhaps, for some larvae that may have entered the roots later than others. Figure 6, D, at 7 days, shows the relatively uniform orientation of the nematodes within the root tissues and the irregular gall formation in the root. Here most of the swelling appears to be in the central cylinder portion of the root, and relatively very little is manifest in the cortex. Distinct increase in size of the nematodes likewise is evident in this picture. In figure 7, A, 9 days, the nematodes are distinctly fusiform and are relatively uniform in size. We note at once that this stage is definitely comparable to the 24-day-old stage in pineapple. It is very evident, then, that development is taking place much more rapidly in the cowpea than in the pineapple. Later stages in the cowpea show it equally strikingly (Fig. 7, B). At 13 days considerable increase in thickness has occurred in some of the nematodes (Fig. 7, C) and at 15 days (Fig. 8, A) a sudden increase in size, manifest in pineapple at 27 days, has already occurred. Note in this picture the irregular outline that, in the fresh root, would appear merely as a slightly swollen, somewhat irregular root. Note, as well, the relationship of the nematodes to such swelling. This and the next picture (Fig. 8, B), 17 days, show particularly well the dark region in the central cylinder where giant cell formation is occurring. Figure 8, C, 19 days, shows the fully developed female nematode. It was in this slide, by careful focusing under higher magnification, that the first egg development was noted. The upper nematode in this picture shows an extruded gelatinous mass in the center of which 4 or 5 eggs were visible. Thus, under the conditions of this experiment, a new generation had commenced by the 19th day. This is to be compared with the pineapple plant growing under identical conditions in which eggs for the new generation were not produced until about 35 days after inoculation. This and the succeeding pictures show, as well, the

extrusion of the egg mass to the exterior of the root. This is well shown in figure 9, A to D, at 21, 25, 26, and 29 days. Figure 9, C and D, shows it particularly well, for individual eggs are discernible in the egg masses.

Figure 9, E, at 30 days, shows a single relatively small gall in the cowpea root containing at least 5 nematodes and with protruding egg masses

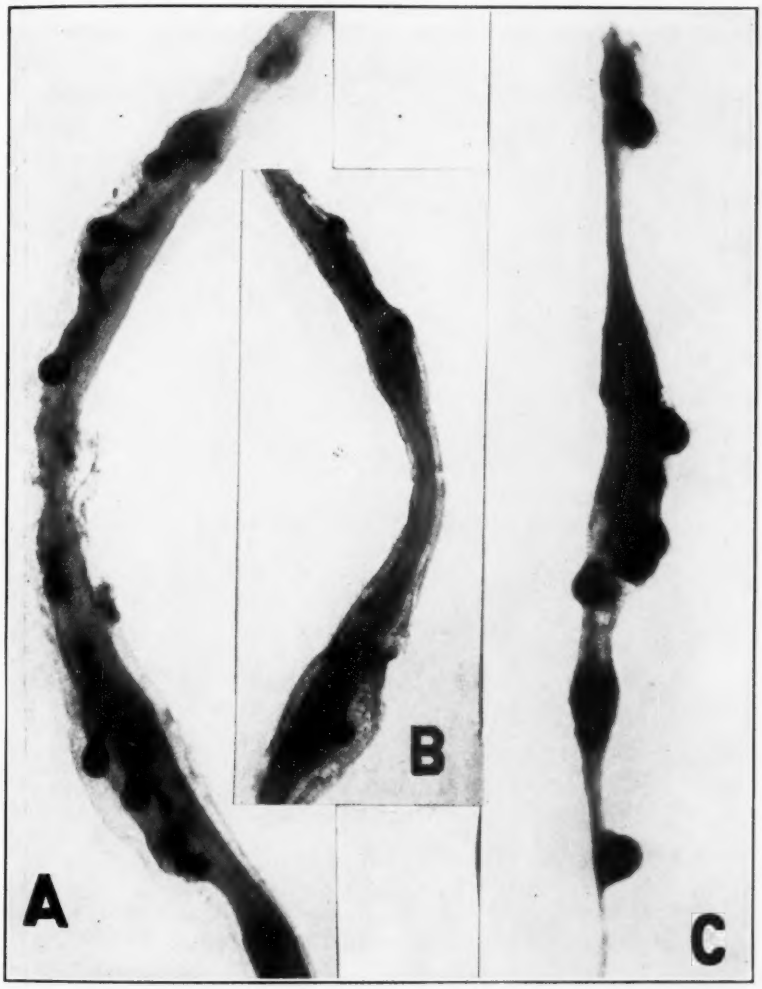

FIG. 8. A. Cowpea root 15 days after inoculation, showing very rapid increase in size of the nematodes after the thirteenth day. Note that the nematodes are completely covered by plant tissues. The stage of their development is not evident from a superficial examination of the fresh root. B. 17 days after inoculation. C. 19 days after inoculation. The upper nematode showed a small quantity of gelatinous secretion in which four or five eggs were to be observed.

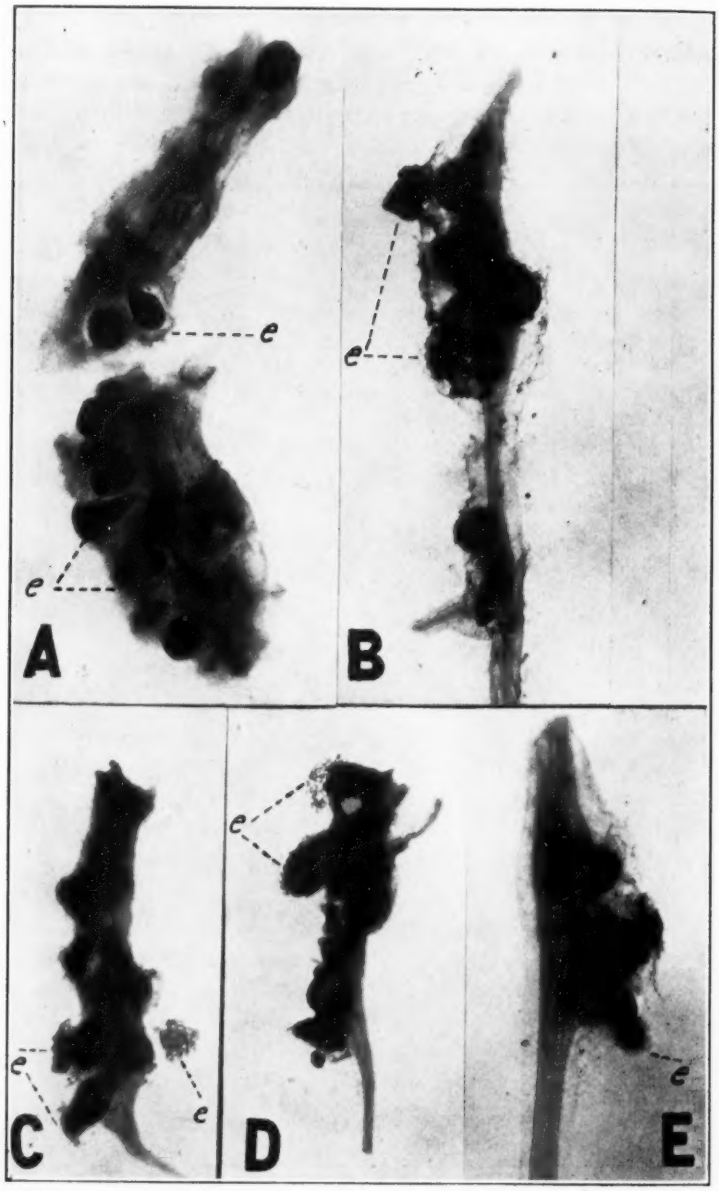

FIG. 9. Cowpea roots. A. 21 days after inoculation, showing several nematodes with extruded gelatinous matrix. B. Another root, 25 days after inoculation. Extruded egg masses are indicated by e. C. 26 days after inoculation. D. 29 days after inoculation. E. 30 days after inoculation. Egg masses with individual eggs evident are to be seen at e in C and D. C to E show egg masses protruding to the exterior of the root.

distinctly evident. This condition is comparable with figure 2 in the senior writer's paper on technique (12) taken directly from fresh material and showing the superficial egg masses. Figure 9, E, shows, as well, the region of giant cell formation in the stele.

Figure 10, A, at 43 days, is presented because it shows, in addition to the adult nematode and large protruded egg mass, the first infection by the new generation of nematodes in the region of root below the old gall. One would judge by the size of the nematodes that this infection is already 4 or 5 days old. The egg mass appears to be entirely empty, the larvae

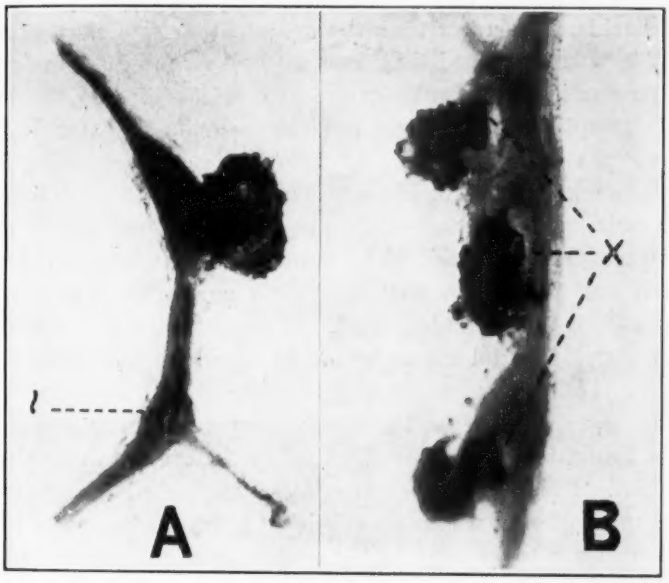

FIG. 10. Cowpea roots. A, 43 days after inoculation. B, 45 days after inoculation. Both show egg masses probably completely evacuated, the eggs having hatched and the larvae migrated. A shows a new infestation of second-generation larvae (1), apparently already 4 or 5 days old. B shows at X the collapsed bodies of dead females, their function in life completed.

probably having already hatched out and migrated away. Figure 10, B, at 45 days, shows the dead and collapsed body of the adult females and the empty egg masses attached to the side of the root. Adherent soil particles make the egg masses somewhat indistinct, but the appearance is typical of what actually occurs.

OBSERVATIONS ON EGG PRODUCTION

Egg production in nematodes within living cowpea roots, first observed on the 19th day, may be considered to have continued until about the 35th

day. Certainly, on the 30th day after inoculation it was still active, as may be seen by examining figure 9, E. On the 43rd day it may be seen from figure 10, A, that the maternal nematode has slightly collapsed and that the egg mass contains no new eggs; therefore, egg production must have ceased sometime prior to this date. Examination of other materials shows that it must have continued until at least the 35th day. This makes a period of about 16 days during which eggs have been produced. The length of this period, too, would seem to depend upon a factor of nutrition. Great variability has been observed between sizes of egg masses on different roots. Under highly favorable conditions egg masses have been observed that are considerably larger than the body of the female from which they came. It is to be presumed that for such heavy reproduction feeding conditions have continued favorable over a long period.

In an attempt to study the egg-producing stage in greater detail heavily infested cowpea roots, excised just before the egg-producing stage was reached, were used. Sections of such cowpea roots, each containing a single mature female nematode, were cut and placed on a white piece of blotting paper on a glass slide and this, in turn, placed in a moist Petri dish. Under these conditions the females continued their normal development and produced eggs for a considerable period. It was possible to make close observations on developmental stages by means of a binocular dissecting microscope.

Females were selected on the first appearance of the gelatinous mass, which was found to appear from 1 to 3 days before the first eggs were deposited in it. This external mass is closely attached to the body of the female in the region of the vulva. Within a day or two after its first appearance eggs are to be seen in the middle portion directly opposite the vulva. From this time on the gelatinous mass increases in size continuously as new eggs are produced and forced into it. The outer portion becomes hardened upon contact with its environment. Consequently, the earliest-laid eggs are confined well within the gelatinous matrix rather than pushed through it by the eggs deposited later. The egg mass adheres to the roots with some tenacity with the result that it becomes tightly packed. If the egg mass is dislodged it is observed that the latest eggs laid are not deeply embedded in the mass and may be separated at a touch.

Within a single egg mass may be found eggs in every stage of development, from the unsegmented forms to hatching larvae. A few eggs in the unsegmented stage were usually found in each egg mass, but eggs were most often deposited after they had reached the 2- or 4-cell stage. Eggs up to the 4-cell stage of development were frequently observed within the ovaries of egg-laying females. Similar observations are referred to by Atkinson

(1) and Stone and Smith (20). Nagakura (15), in his recent excellent paper on the embryology of *Heterodera radicicola*, does not mention it.

During the course of this study on egg deposition 7 females were under observation for a 10-day period. During this period the eggs deposited daily were removed from each female and counted. At the end of the 10-day period the bodies of the females were opalescent or nearly transparent, with the empty ovaries adhering to the body walls. In this condition their appearance contrasts sharply with that of females before eggs are deposited; at this time the body is completely white and opaque. A tabulation of the results of this observation is given in table 1.

TABLE 1.—*Egg production from Heterodera females in excised roots during 10-day period*

Female number	Days										Average
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	
1	10	32	32	45	45	40	32	28	10	14	28.8
2	17	28	43	40	44	35	30	26	20	23	30.6
3	15	22	18	28	41	42	34	8	12	10	23.0
4	26	36	15	20	34	43	70	13	30	7	26.4
5	25	12	38	30	30	43	35	12	0	10	23.5
6	27	38	51	34	23	29	17	19	8	12	25.8
7	8	12	26	31	33	46	22	23	21	12	23.4
Average	18.3	25.7	31.9	32.6	35.7	39.7	34.3	18.4	14.4	12.6	25.9

It is to be recognized that these results are not comparable to what takes place in nature, inasmuch as the roots were not growing normally during the period of observation. Nevertheless, the fact that egg deposition varies from 23 to 30 per day in different individuals for the 10-day period, with a general average of 26 per day for the lot, is decidedly interesting. It is highly significant that mature females may continue to develop eggs for a considerable period, even though the root in which the female is embedded has been cut from the parent plant. This fact is of real importance in its relation to the use of the trap crop for nematode control.

Under normal conditions in the growing roots a female nematode may produce several times the number of eggs indicated by this table. During the progress of the present studies many egg masses were counted with between 600 and 800 eggs. As many as 1,200 eggs were counted from one mass, showing evidence of eggs that had already hatched and, likewise, evidence that new eggs were still being deposited at the time the egg mass was removed. Such large egg masses were to be found in both pineapple and cowpea roots. These figures are very much higher than those ordinarily given for numbers of eggs in *Heterodera radicicola*. The figures most commonly encountered in the literature are between 300 and 500 from Bessey

(3), Childs (5), Frandsen (7), and Newhall (17), 300 to 600 from Nagakura (15), while, from Africa, comes the low figure of 60, reported by Sandground (18). This difference is significant, of course, in that it makes evident the very rapid rate of reproduction under Hawaiian conditions.

DISCUSSION

The root-knot nematode is an obligate parasite, though it may remain alive for long periods in the soil in either the larval or the egg stage. It does not develop outside the roots of the host plant; consequently, the host is requisite for it to go through its life cycle. One of the most significant developments from this study has been the demonstration that, besides temperature, the host plant itself plays an important part in influencing the length of life history of the root-knot nematode. While the period required for penetration and the 1 or 2 days' migration period are approximately identical in cowpea and pineapple, the period required for development to the uniform fusoid form, common to males and females alike, is considerably longer in the pineapple than in the cowpea. The former required approximately 24 days for this, whereas, in cowpea, this stage was reached in only 8 or 9 days. The 27th day in pineapple is about the same as the 14th day in cowpea, showing that the time between the fusoid form and the beginning of rapid development of the female was approximately the same for both host plants. The first eggs were not observed until about the 35th day in pineapples, whereas they were seen on the 19th day in cowpea. This indicates again slower development in the later stages in the pineapple root than in the cowpea root. Table 2 is a condensed comparison of lengths of time required for developmental changes in the two host plants.

TABLE 2.—*Comparison of length of life history of Heterodera radicicola in pineapple and cowpea*

Stage of development	Time from inoculation	
	Cowpea	Pineapple
Penetration	6 to 48 hours	6 to 48 hours
Migration ends	1 to 3 days	1 to 3 days
Fusiform stage begins	9 days	24 days
Rapid growth of female begins	13 "	27 "
First egg production	19 "	35 "

It is evident from this table that the greatest difference between the two plants lies in their influence on length of time required for the development from larval stage to fusiform stage. This may be a matter of nutrition. Possibly it is related to development to the fullest extent of the multi-

nucleate giant cells, the so-called nourishing cells (*cellules nourricières*) of Molliard (14) that appear to act very much like nectaries in providing a continuous supply of nutrient materials for the continued growth of the nematode. When once fully developed further growth and increased capacity for reproduction (volume of egg development) on the two plants do not differ materially.

These exact results on difference in length of life history corroborate very nicely the opinion of Bessey in 1911 (3) that "Time required for the development from the egg to the mature egg-laying individual depends to a great extent upon the temperature *and upon the plant affected.*" Our several years' observations likewise verify Bessey's opinion with regard to the influence of temperature. The life history is very much longer in winter than in summer, even in Hawaii, where growing conditions prevail throughout the year. Miss Tyler's results (22) appear to be exact with regard to the relation of temperature to length of life history. In the light of our results, however, it is certain that her figures as to length of life history apply only to the particular host plant with which she was working. The minimum and maximum figures she has reported are 17 days at 27° C. and 63 days at 15½° C. Various other investigators have given length of life history in round numbers and without relation to temperature. During the period of our studies the air temperature of the greenhouse in which the observations were made ranged from 20° to 33° C. In all probability the soil temperature was near the optimum point for activity of the nematode the greater part of the time. As stated before, any absolute figures would require controlled temperature conditions.

The minimum length of life history might be considered to be the length of time that has elapsed from the time of inoculation to the hatching of the first eggs from the egg masses that developed as a result of this inoculation. Since, under the conditions of this experiment, the first eggs produced, 19 days after inoculation, required approximately 5 days to hatch, then, under these conditions, the length of life history was about 24 days. If these same conditions were to prevail throughout the year, then, with cowpea as the host plant, there might be said to be the potential possibility of 15 complete generations during the year. That this figure is far in excess of what actually occurs in Hawaii is evident from the known fact that life histories are very much slowed up in the winter months.

It should be noted that under ordinary field conditions nematodes are not to be found at any one season solely as eggs or as larvae or as developing organisms within roots of established host plants. Inasmuch as (1) length of time from larva to adult varies greatly within the same root, (2) eggs are produced during a period of approximately 2 weeks; and, finally, (3) they hatch during a period of several days immediately after, even the sec-

ond generation of nematodes is to be found in all stages in the field. Thus, the possibility of attacking nematodes at a season when one might expect an inherently weak stage in their life history, as is often done with insects, is eliminated from consideration in attempts at control, except by the trap crop method, before the first individuals have produced eggs. A pineapple root, typical of what actually occurs in the field, is shown in figure 11, A. Here, infections in the slow-growing root have apparently been continuous through at least 40 days, as nematodes in all stages of development are to be seen in the same root.

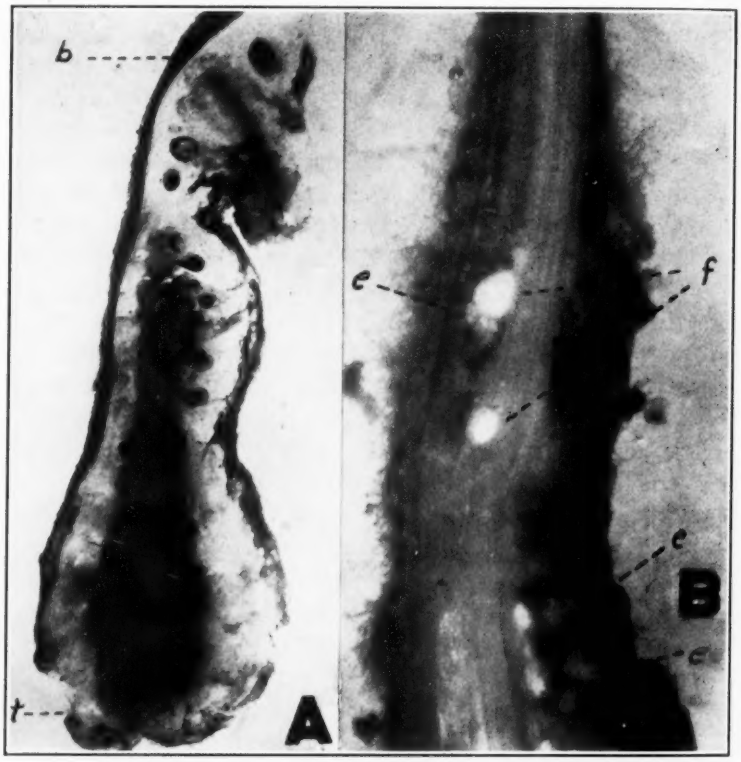

FIG. 11. A. Pineapple terminal gall taken directly from the field and processed by the usual method, showing all stages of development of the nematodes within the same root. In the newer terminal portion, t, larvae have only recently entered; in the older basal portion, b, are to be seen mature egg-laying females. About × 8. D. Longitudinal section of an uncleared root from the field showing occasional female nematodes, f, and egg masses, e, entirely buried within the root tissues. About × 16.

Various early investigators, particularly Garman (9), Neal (16), Massee (13), and Frandsen (7) apparently have mistaken the dead body of the

female filled with developing eggs for the gelatinous egg-containing matrix. Likewise in this connection, Frank (8) says ". . . die befruchteten Weibchen zu birn—oder flaschen förmigen bis ½ mm groszen *eiererfüllten Cysten* anschwellen." Nagakura (15) makes clear the true nature of this body and refers to Voigt (23) (whose paper was not available to us) as having done so as early as 1891. At later stages this enclosing mass becomes thickened and brown and may be confusing because of its cyst-like nature. Indeed, this egg mass does function to some degree, as a cyst, in that it serves to protect the contained eggs and to hold them over for a considerable period during unfavorable conditions.

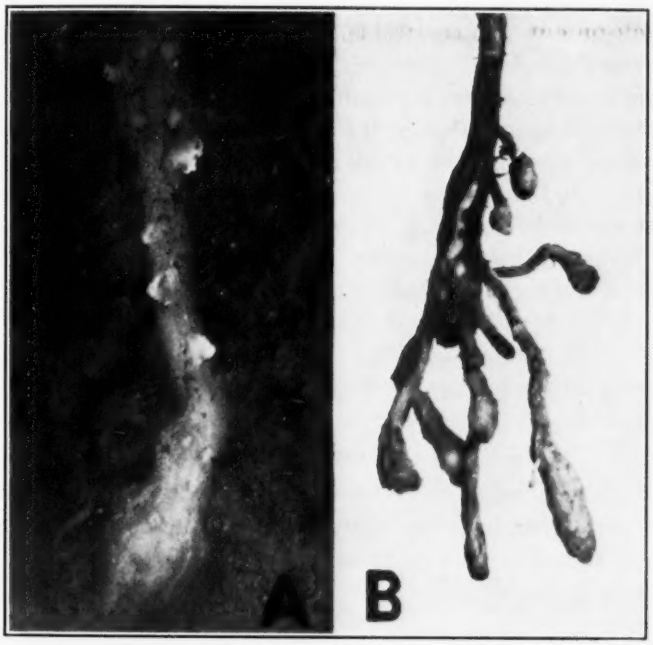

FIG. 12. Pineapple root knot. A. Case with protruding nematode egg masses. About × 4. B. Much-branched root terminus, each branch with a typical terminal gall in which the egg masses are mostly borne internally, as in Figure 5, C. About natural size.

It is to be noted that the breaking through of egg masses to the exterior of the root is of considerable significance in the propagation of nematodes in the field. These eggs begin to hatch almost immediately under favorable conditions. Larvae are quickly released directly into the soil and are available for infesting such other roots as are in the immediate vicinity or as may be reached by the larvae in their migration under favorable conditions through the soil. This condition is to be contrasted with that shown in the

pineapple (Figs. 5, C, and 11) in which either slow migration of hatched larvae through the root tissues to the exterior or decomposition and breakdown of the root are necessary before the larvae reach the soil for infestation of new roots. In many cases this undoubtedly takes several months. It is a condition, of course, which provides protection of the nematode offspring through a longer period, but, at the same time, retards tremendously the propagation of new generations.

It is not to be concluded from this account that these two types of egg-mass development are invariable with the two host plants. Actually, both types occur with both host plants. Figure 12, A, shows external egg-mass development in the pineapple as compared with the more typical condition of terminal-gall development, figures 12, B, and 11, with egg masses found in the interior.

Observations on time and manner of penetration indicate that, where there is a very large number of larvae present, penetration itself is likely to take place quickly, rather than otherwise, and to be complete so far as immediately available larvae are concerned. While there must be a first invader, as shown by figure 6, A, in cowpea, still, mass action appears to play a part in the heavy invasion immediately following. Figure 1, C, shows a large number of nematodes entering the roots at approximately the same point in preference to other available portions of the root-tip tissues. The resistance of surrounding soil particles, with consequent opportunity for the larvae to employ pressure, undoubtedly plays a part in their ultimate entrance.

SUMMARY

Cowpea and pineapple roots were inoculated by placing large numbers of *Heterodera radicicola* larvae at the tips of young actively growing roots. At 6, 12, 18, and 24 hours and at daily intervals thereafter, root tips were excised and immediately killed with Flemming's solution (which, by virtue of the contained osmic acid, stained the nematodes black) after which they were washed, gradually dehydrated through the alcohols, and finally cleared in clove oil. By this process the nematodes could be seen plainly in their natural positions in the root tissues in all stages of development from first penetration to maturity and the beginning of egg production. The root materials so preserved were used for making permanent slides and for photographic reproduction. The principal observations made thereon are recorded as follows:

1. The first larvae penetrate the root in less than 6 hours and penetration continues for at least 24 hours. Mass action appears to be a factor in heavy penetration of root tips. Large numbers of larvae often enter the root at or near the same point. Penetration is most abundant in the

meristematic region of the root tip beside the root cap, though entrance sometimes occurs through the root cap, itself.

2. Migration of the larvae occurs during a period of 2 or 3 days. This migration ultimates in locating a definite tissue in the maturing part of the root.

3. On or near the 4th day the larvae become definitely oriented within the root tissues. The head end becomes established in the cells of the endodermis of the cortex or in the outer periphery of the stele. The body lies in the cortex with the extremity lying diagonally towards the root tip. Thus oriented, they remain in this position, the females permanently, the males until the final molt at maturity.

4. Forward root growth is retarded within the first 24 hours after penetration. In cases of extremely heavy infestation, growth is permanently checked. In lighter infestation, after a period of retarded development, renewed growth, brought about by renewed activity of the meristematic tissues, often occurs. This is somewhat rare in pineapple root tissues but is the usual thing with cowpea.

5. The length of time elapsing from initial inoculation to the first egg development was, in cowpea, 19 days, in pineapple, 35 days. This difference appears to be inherent in the two host plants.

6. Egg masses in cowpea roots frequently break through to the exterior of the root. Here they are in such a position that the eggs often begin to hatch immediately, thus releasing a new generation of larvae into the soil for ready infestation of new roots. In pineapple roots egg masses are usually produced in the interior of the gall; thus release of the new generation of larvae into the soil is delayed and, consequently, the life history is still longer.

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INVESTIGATIONS ON APPLE BLOTCH[1]

EDWIN J. KOHL[2]

INTRODUCTION

Apple blotch, a disease of the cultivated apple (*Malus Malus* (L.) Britt.) and various species of native crab apples, is caused by the parasitic fungus *Phyllosticta solitaria* E. & E., which attacks the fruit, leaves, twigs, and buds of its hosts. It was first reported by Underwood (22), who collected it on the leaves of *Pyrus coronaria* L. near Crawfordsville, Indiana, in 1893. Underwood submitted this material to Ellis (5), who described and named the organism. Subsequently the disease has been reported from nearly every important apple-growing region east of the Rocky Mountains and south of 40° north latitude and has been made the subject of numerous investigations. Most of the extensive literature that has resulted is cited and reviewed in the publications of Scott and Rorer (20), Lewis (16), Roberts (19), Gardner, Greene, and Baker (9), and Guba (12). It, therefore, seems unnecessary to include a full review of the literature in the present paper. Such references to previous work as seem pertinent will be made in connection with the appropriate topics below.

Notwithstanding the excellent contributions that have been made by earlier workers, certain important gaps remain in our knowledge of blotch. Much difficulty has been encountered in tracing the development and maturation of spores in nature and no satisfactory technique has been reported for the consistent production of viable spores in culture. Infection experiments have consequently been seriously hindered, and many essential phases in the progress of disease improvement have remained obscure. In the present work, therefore, the attempt has been made to gain a more adequate understanding of the production of viable spores, both in nature and in culture, and of the phenomena of infection and disease development.

[1] Joint contribution from the Department of Biology, Purdue University, and the Department of Botany, Purdue University Agricultural Experiment Station, La Fayette, Indiana. The results of these studies were presented to the faculty of the University of Wisconsin in partial fulfillment of the requirements for the degree of doctor of philosophy.

[2] The writer wishes to acknowledge his indebtedness to Dr. G. W. Keitt, University of Wisconsin, for suggestions and supervision in the prosecution of these studies; to Dr. M. W. Gardner, of the Purdue University Agricultural Experiment Station, for suggesting this problem and for active cooperation throughout the progress of the work; to Dr. H. E. Enders, Head of the Department of Biology, Purdue University, for much encouragement and stimulation throughout these studies; and to Prof. C. L. Burkholder, Department of Horticulture, Purdue University Agricultural Experiment Station, for field facilities and assistance.

SPORE PRODUCTION AND MATURATION IN CULTURE

The production of spores in artificial culture has long been known to be difficult of accomplishment. Scott and Rorer (20), Lewis (16), and Roberts (19) report production of spores on sterile apple twigs. Scott and Rorer state that sporulation was also brought about on corn-meal agar, but Roberts reports no sporulation on this medium. Guba (12) calls attention to the difficulties he experienced in obtaining fruiting cultures on any medium. He reports an instance of sporulation on corn-meal agar 7 days after transplanting the fungus but makes no statement as to whether the fungus was newly isolated in this instance. At another time, when sporulating cultures were obtained, he states that the age of the canker and the season of the year may have had some influence on fructification. He observed that subcultures never sporulated.

In the writer's studies, also, much difficulty was experienced in bringing the fungus to sporulation. A wide variety of artificial culture media was used. Corn-meal agar was used for the isolation of the fungus from fruits and twigs because of the ease of preparation of the medium and the possibility that the pycnidium-like bodies that are abundantly formed upon it might produce spores. The corn-meal agar contained 50 gm. of whole white corn meal and 20 to 25 gm. of agar per liter. The hydrogen-ion concentration of this medium was pH 5.3 to pH 5.5.

At various times it was observed by means of a binocular microscope that, in tube cultures produced by transfer of noncontaminated infected tissue from blotched Rome apple fruits, pycnidia were produced over the whole thallus. These pycnidia tended to round off and become somewhat spherical. When such cultures were incubated at 19° C. spores were found approximately 20 to 22 days after transfer. They did not germinate at once, a period of maturation seeming to be necessary. These results are similar to those of Guba (12), who notes that spores from culture sowed in water germinated only after some time had elapsed. After further trial it was found that, on this medium, the fungus in the original cultures isolated by tissue transfers from storage fruits of the Rome variety consistently produced fertile cultures (Fig. 1, A and B). Neither the use of whole yellow corn meal instead of whole white corn meal nor the use of tap water of 22 grains hardness instead of distilled water caused any apparent difference in results. New isolations were repeatedly made by this technique and the resulting cultures, which contained the transplanted host tissue, sporulated consistently.

Because lateness of the season prevented trial with other varieties and because Rome fruits presented clean smooth lesions, storage fruits of only this variety were used extensively. In earlier work cultures were made by this same technique from young apple fruits collected in late summer and

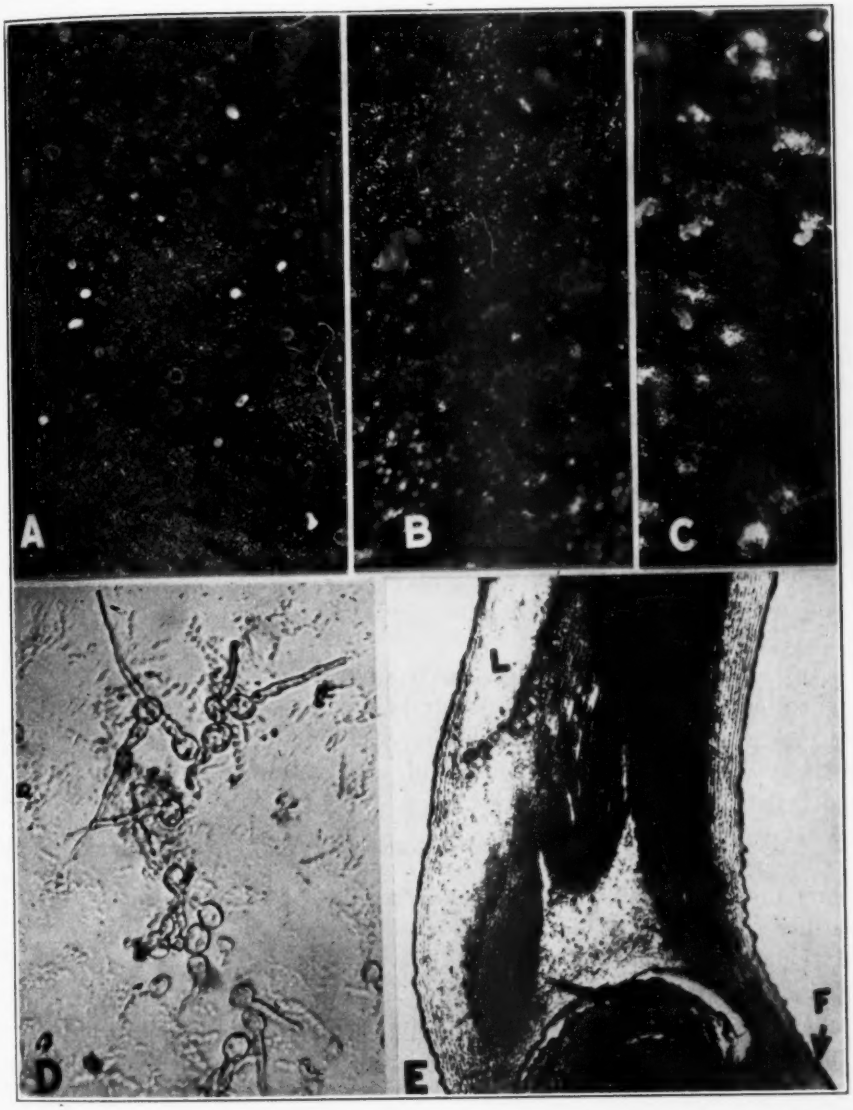

FIG. 1. A. A culture of the apple-blotch fungus on corn-meal agar, showing spores oozing from the fertile pycnidia. ×9. B. Same as A. ×25. C. Spore horns or cirri of the blotch fungus, extruded from the pycnidia on apple twigs, after being atomized in a moist chamber. ×15. D. Photomicrograph of living blotch spores from natural sources, germinated in water on a glass slide, 18 hours after sowing. ×150. E. Longitudinal section of petiole of apple, showing a blotch lesion on the left (L) and a bud. The mycelium of the fungus was traced into the abscission layer at F. ×25.

early autumn as well as from diseased twigs, but no sporulation was observed. Much contamination occurred in cultures from diseased twigs.

As was reported by Guba (12), subcultures obtained by making mycelial transfers failed to sporulate. No sporulation was obtained from transfer of spores or mycelium to sterile apple twigs in test-tubes. Similar subcultures on a variety of other media also failed to sporulate.

The data that have been presented strongly suggest that suitable living host tissues may influence sporulation. The exact nature of this influence has not been determined.

Guba (12) records spore production on artificial media at temperatures of 25° and 30° C. While no comprehensive studies regarding relations of temperature to spore production were undertaken by the writer, it was observed that certain cultures sporulated abundantly at 19° to 21° C., whereas parallel cultures at 28° C. failed to fructify. Temperatures of 19° to 21° C. seem to be very favorable for sporulation.

SPORE PRODUCTION AND MATURATION IN NATURE

Spore production and maturation in nature have been given some attention by Roberts (19), who reports a progressive maturation of blotch spores from natural sources, as determined by sowings made in water at daily intervals during May.

In the present work, germination studies were undertaken during several summers with spores from natural sources and from cultures. Many sowings were made in various dilutions from 0.1 per cent to 1 per cent of citric acid, hydrochloric acid, nitric acid, sodium oxalate, hydrogen peroxide, and boiled and fresh decoctions of apple fruits, apple leaves, apple bark, prune decoctions, and sugar solutions. Control sowings were made in sterile distilled water. In many instances similar tests were made with spores that had stood, without germinating, for 24 hours in drops of sterile distilled water. No instance was found in which the presence of any of the solutions used stimulated germination. Ether, chloroform, and carbon dioxide were liberated over sowings of spores in sterile distilled water, with negative results. When spore germination did result it was uniform within a small percentage for the spores contained in a single pycnidium. Because of the large percentage of spores that did not germinate during June, it is thought that not all pycnidia mature their spores simultaneously.

In order to ascertain at what time of the year spore formation and maturation occur under natural conditions another type of experiment was undertaken. Twigs with cankers, collected at Mitchell and La Fayette, Indiana, were brought into the laboratory at various times. Such twigs were placed upright in a moist chamber at approximately 22° C. and atomized daily with distilled water to cause the extrusion of spores. The

extruded spores had the form of spore horns or cirri (Fig. 1, C). The extrusion of spores was used as an approximate index to spore maturity. The results of these experiments, which appear in table 1, show that, from

TABLE 1.—*A summary of results of experiments relative to morphological and physiological maturation of spores of Phyllosticta solitaria in twig cankers in 1927*

Place and date of collection	Spores extruded after stated intervals in moist chamber	Germination of extruded spores on corn-meal agar
Mitchell, Ind.		
January 20	3 weeks	None
February 8	24 days	"
" 15	18 "	"
March 4	15 "	"
" 10	9 "	"
" 30	4 "	"
April 1	3 "	12 days after sowing
" 5	24 hours	18 " " "
" 20	8 "	5 " " "
" 27	5 "	5 " " "
La Fayette, Ind.		
May 5	3 "	3 " " "
" 15	3 "	3 " " "

twigs collected early in the season and placed in a moist chamber, spores were extruded only after a relatively long period of exposure to moist conditions. As the time of year approached when infection occurred under natural conditions, the interval between placing of the twigs in the moist chamber and extrusion of spores became progressively shorter. The interval between sowing and germination of the spores also shortened continuously. The extruded spores had every morphological indication of maturity. The lack of germination, however, indicates that they were not physiologically mature. No germination occurred in collections made before April 1. It was only after the spores had gone through a sufficient period of rest or afterripening that they germinated. The results of these studies would indicate that under field conditions as well as in culture there is an interval between the morphological and physiological maturity of spores. The period between the time of complete morphological maturity and physiological maturity was not successfully shortened by artificial means. In southern Indiana the spores, ordinarily, are morphologically mature by about April 1 and physiologically mature by May 1.

SPORE-GERMINATION STUDIES

Relations of moisture prior to germination. Considerable observational and experimental evidence suggests that the viability of spores may be

affected by the conditions of moisture to which they are subjected before germination. It is conceivable that the common lack of viability of spores in culture may be attributable in part to injury by drying, since it requires approximately 35 days for the spores to mature in tube cultures. Spores collected in the field after protracted dry periods and sowed on slides showed a much lower percentage of germination than those produced and naturally matured during moist weather. Evidence of injury to spores by desiccation was also obtained from field-infection studies that are herein reported. It is shown in figure 3 that, at La Fayette, Indiana, in 1925, a long period of drought followed petal fall. Infection did not occur after this period until 6 rains had fallen, the 3rd of which amounted to nearly an inch. The sixth of these rains, during which infection occurred, was 0.6 inch. While such prolonged dry periods are infrequent, it is a matter of common observation and knowledge that during a dry season infection is relatively light, while during a wet season infection is quite heavy. Reference to figures 6, 7, 8 and 9 will illustrate how uniformly infection occurs during a continuously wet season.

Experiments were undertaken to determine the effect of drying on the germination of spores. During June and July spores were placed on bits of sterile cover glass and dried in the open air in the laboratory for periods of 1, 5, 10, and 30 minutes and 1, 2, 4, and 6 hours, respectively. None of the spores that were dried longer than 2 hours germinated on corn-meal agar. Spores that were left continuously in water as controls germinated vigorously within 12 to 24 hours. Numerous replications of this experiment gave similar results. These results, in conjunction with lack of natural infection when rains followed protracted dry periods, suggest that the viability of spores is sharply reduced by desiccation.

Adhesion of spores to substratum. The presence of some substance or envelope causing spores to adhere to their substratum was suggested by Voges (23) in germinating conidia and ascospores of *Venturia inaequalis* (Cke.) Wint. Keitt and Jones (15) made further studies of the phenomena of adhesion of the spores of the same fungus. They found that ascospores of Venturia exhibited a marked adhesion to the substratum within 15 minutes after they were sown in sterile distilled water on glass slides and that in 30 minutes 50 per cent of the spores were not washed off when the slide was held for 20 seconds in a strong stream of water falling from a faucet. Conidia of Venturia exhibited a similar marked adhesion. These investigators did not succeed in demonstrating the presence of an envelope of the type suggested by Voges.

Ordinarily, when mature spores of *Phyllosticta solitaria* are sown in distilled or tap water on physiologically clean glass slides, germination proceeds as has been described by Guba (12). It was observed in the

present writer's studies that at some time between sowing and the first appearance of a germ tube the spore becomes firmly adherent to the slide. This was demonstrated by placing the slide at an angle of 45° under a faucet attached to the city water system (pressure 50 to 70 lbs.) and allowing a stream of water of approximately 12 mm. diameter to fall upon it for 15 seconds. The germinated spores were still attached to the slide, whereas the spores that had not germinated were washed off. By inducing strong currents in open water mounts under microscopic examination it was clearly evident that both the spores and portions of their germ tubes were firmly anchored to the glass.

GERMINATION AND APPRESSORIUM FORMATION ON LEAVES

The germination of spores on leaves was undertaken with a view to determining the mode of entrance of the fungus into the host. By placing cankered twigs in a moist chamber and atomizing them with water, the extrusion of spores was effected within a few hours. Spores thus obtained were sown upon marked wet areas of detached leaves that were placed in a moist chamber. Germination was very sparse and erratic. Consequently, a different method was adopted. A small portion of a relatively clean canker bearing several pycnidia was cut away and soaked several minutes in water. It was then placed so that the ostiolar side of the pycnidia rested upon marked wet areas on the upper surfaces of relatively young clean leaves. Only distilled water was used in wetting the leaves. At intervals of 12 to 72 hours after inoculation the marked areas of the leaves were cut away, killed, and fixed for 24 hours in equal parts of 95 per cent alcohol and glacial acetic acid. They were then cleared in a saturated solution of chloral hydrate (Peace, 17), after which they were washed in water, stained in cotton blue or anilin blue, and mounted in glycerin. The material thus prepared was not suitable for imbedding in paraffin. When paraffin work was anticipated, formalin acetic-alcohol (Rawlins, 18) was used as a fixative and glycerin as a clearing agent, since glacial acetic-alcohol as a fixative followed by chloral hydrate as a clearing agent caused excessive hardening.

Spores that germinated on leaves differed from those that germinated on slides in that they formed appressoria. Appressoria have not been seen on slides. The germ tubes of spores sown in water on slides merely proliferated and branched profusely in a very short time (Fig. 1, D). On leaves, appressoria were formed in 20 to 30 hours (Fig. 2, A and B).

The term appressorium was used by Frank (6) in his studies of *Fusicladium tremulae* Fr. Appressoria have since been noted in a wide variety of fungi by many workers. They have been studied by de Bary (2) in Selerotinia, Halsted (13) in species of Gleosporium, Aderhold (1) in *Venturia inaequalis*, and others. The writer has found no record of appressorium formation in other Phyllosticta species.

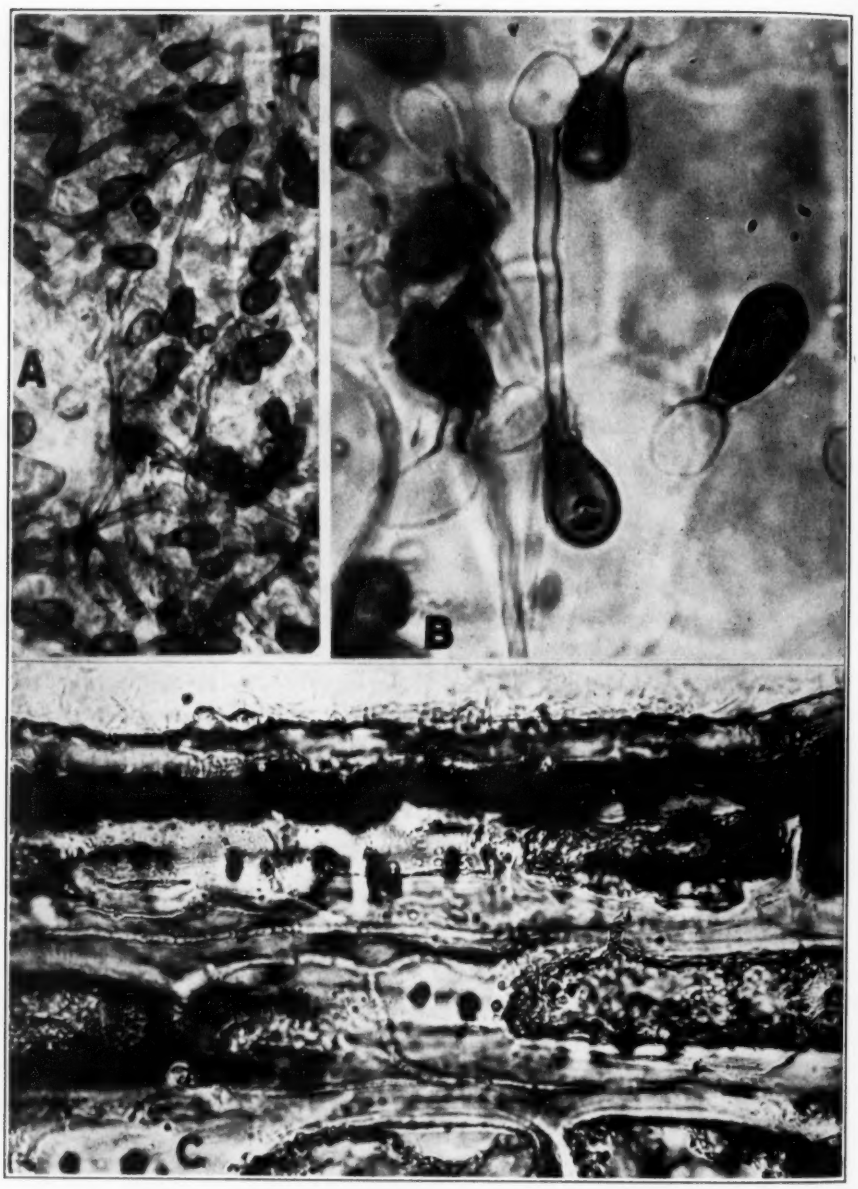

FIG. 2. A. Dark appressoria of spores of the apple-blotch fungus from natural sources, germinated on an apple leaf. ×200. B. Same as A except that the photomicrograph was made with oil-immersion objective. Empty spores and germ pores of the dark appressoria are better resolved. Epidermal cells of the apple leaf are seen faintly below the appressoria. ×800. C. Intercellular mycelium of *Phyllosticta solitaria* in collenchyma of the petiole, fixed in Schaffner's chromo-acetic fluid and nonstained. Septate hyphae may be seen. ×650.

The appressoria of *Phyllosticta solitaria* are olivaceous brown. They usually are 10 μ long by 6 μ wide and are pear-shape to egg-shape in outline. Sometimes they are shaped much like a boxing glove. When first formed they contain a densely granular material. Later, a germ pore in the basal wall is quite evident and the content of the appressorium is less dense. Measurements and observations were made by means of a 1.40 N. A. oil-immersion objective. The germ tubes have the same olivaceous brown color as the appressoria and are from 4 to 14 μ long by 2 μ wide. Hyaline germ tubes have been observed on leaves and, in such cases, no appressoria were formed. It is of interest to note that spores germinated on glass slides form hyaline germ tubes and no appressoria. In all cases observed walls of the empty spores were hyaline and very difficult to see. It was only by the aid of an oil-immersion objective that they could be seen at all. In contrast, the dark germ tubes and appressoria were easily seen.

Mode of penetration. It was concluded by Blackman and Welsford (3) that the direct penetration of the cuticle of host plants by germ tubes or infection hyphae of parasitic fungi is a mechanical process and in no wise dependent upon local dissolution of the cuticle. This conclusion was made as a result of germination studies with *Botrytis cinera* Pers. They found that an appressorium of Botrytis can mechanically force a germ tube through a thin layer of gold leaf. Brown (4), however, states that a powerful enzyme is formed that can cause disintegration of the cell wall and the middle lamella and permit infection hyphae to penetrate the host cells. Gardner (7) found that *Colletotrichum lagenarium* (Pass.) Eriks. & Henn. also formed appressoria but that a distinct swelling of the cell wall was sometimes present at the point of contact between the appressorium and the cuticle, as seen in cross sections. In the present studies with *Phyllosticta solitaria*, the details of host penetration are not known beyond the formation of an appressorium. A germ pore can be seen in the basal wall of the appressorium, as the photomicrographs show. Attempts to reveal the details of penetration by means of paraffin sections have thus far been unsuccessful due chiefly to hardness of the material.

INOCULATION STUDIES

Successful inoculation and infection of apple trees with a suspension of spores of *Phyllosticta solitaria* from natural sources was reported by Scott and Rorer (20) and Roberts (19). Guba reports that his attempts to induce infection failed. The writer tried similar experiments by spraying young leaves and twigs with spore suspensions, but no infection resulted.

Further experiments were undertaken, therefore, to bring about infection. It was assumed that failure of earlier trials resulted from an excessive drying of spores. The method employed in these experiments is essen-

tially that previously described in this paper for the germination of spores on leaves. In a first attempt, small bits of cankers with pycnidia, previously soaked in water, were placed with ostiolar side in contact with the upper surface of the leaves and on petioles of potted apple trees. A piece of moist cotton was placed about each inoculated area and removed 24 hours later. In a second experiment, the inoculated twigs were enclosed in large test tubes plugged with moist cotton. In a third trial, in which moist chambers were used, as in the second experiment, spores from corn-meal-agar cultures were substituted for the natural inoculum. Controls without the use of fungus inoculum developed no infection. In a fourth test potted disease-free Duchess apple trees, similar to those used above, were placed beneath an old diseased tree until a good rain had fallen, so that extruded spores carried in the drip from twigs might fall upon the potted trees. With each of the four methods, petiole lesions of the disease were produced. The symptoms were typical of naturally occurring apple blotch. The fungus in each case was reisolated in pure culture by tissue transfer and showed cultural characteristics typical of *Phyllosticta solitaria*. Cultures of the reisolated fungus have not yet sporulated.

PATHOLOGICAL ANATOMY AND RELATION OF FUNGUS TO THE HOST TISSUES

The pathological anatomy of petiole and fruit lesions and cankers has recently been described by Guba (11). The present writer has made similar studies with results that have been generally confirmatory. Some exception to Guba's studies of the pathological anatomy of the canker, however, may be taken regarding the origin of the abnormal parenchymatous tissue beneath the phellem. He attributes this exclusively to the cambium, whereas the present writer has observed its origin from other tissues, notably the secondary phloem. Whether this abnormal tissue should be termed phelloderm is open to question.

Comparatively little attention has hitherto been paid to the progressive development of the fungus following host penetration and the relation of the fungus to the host tissues. Increasing understanding of the importance of petiole transmission, of which there has been much evidence from field and cultural studies of Gardner (8), emphasized the desirability of giving further attention to this phase of the subject.

Attention was given to methods of killing, fixing, and staining the fungus in order to permit a more rapid and satisfactory examination of histological sections. Material was killed and fixed in Gilson's fluid and stored in 80 per cent alcohol. Sections cut by the freezing method were placed in a 1–1000 gold chloride solution for about 30 minutes. The host cells took on a faint purplish color and the mycelium became granulated black. A fair degree of differential staining was obtained. This is the

method that was employed by Miss Gerry (10). The use of aniline stains or haematoxylins requires such heavy overstaining that the host tissue, in thick sections, obscures the mycelium. No staining at all is much preferable to such heavy overstaining for studying the mycelium in the host or tracing it across the abscission layer. It is notable that young mycelium stains with difficulty and older mycelium is very resistant to nearly all stains. Older mycelium can be traced rather easily because of its olivaceous brown color.

After the fungus has penetrated the host, it may be found in an intercellular position, as mentioned by Guba (11). In the petiole it limits itself to the collenchyma (Fig. 2, C). Although hundreds of sections were examined, the fungus was not found in any tissue deeper than the collenchyma. It could be traced a number of millimeters, longitudinally, in the collenchyma beyond the visible margin of the petiole lesion. Particular attention was given to tracing it on the side of the lesion toward the abscission layer. It was traced successfully, though with difficulty, through the abscission layer, so that we have microscopic evidence of petiole transmission of the fungus into the bark, where it may overwinter. Once in the bark, the fungus becomes established in the collenchyma.

Petiole transmission occurs during July or August, depending somewhat upon how early or how late infection has taken place. At any rate, in the vicinity of Knightstown, Indiana, as reported by Gardner (8), or La Fayette, Indiana, cankers infrequently appear in the autumn as the result of petiole transmission except where the lesion of the petiole was close to the bark and formed a small bark lesion directly. Such cases are rather uncommon. The mycelium in the bark is always in advance of the macroscopically visible edge or margin of the canker. The mycelium can be found any month in the year considerably in advance of the lateral edges of the cork tissue of which successive zones are formed in the cortex under the invaded collenchyma. The advancing mycelium can be traced readily through apparently normal tissue by the fact that the host cells show a yellowish or yellowish brown discoloration along the line of mycelial advance, except near the very tip of the most advanced hypha.

The relation of the fungus to the tissues of the fruit is of interest because of the extreme difficulty in locating the mycelium. This difficulty arises from the fact that the advancing hyphae are closely appressed to the walls of the host cells. A brownish discoloration of the host cells as a result of their reaction to the fungus almost completely obscures the fungus.

Petiole transmisson of the fungus has been definitely shown by Gardner (8) to be the principal means by which the fungus gains entrance into the bark of bearing wood. He found that a majority of the new cankers appearing in early spring occur at or near a leaf scar. By means of tags he demonstrated that such cankers appeared where a diseased petiole had

been attached the previous season. A similar case of petiole or leaf transmission of a fungus into the bark is that of *Cronartium ribicola* Dietr. on *Pinus strobus* L. as reported by Tubeuf (21). Hedgcock (14) finds also that, on Populus, petiole transmission is the means by which *Dothichiza populea* Sacc. & Briar. gains entrance to the bark for overwintering.

TIME OF INFECTION IN THE FIELD

For the study of the time of natural infection at La Fayette and Mitchell, 1-year-old Duchess apple trees were planted in 8-in. flower pots. These were sunk in rows some 400 feet from the old diseased trees. Either

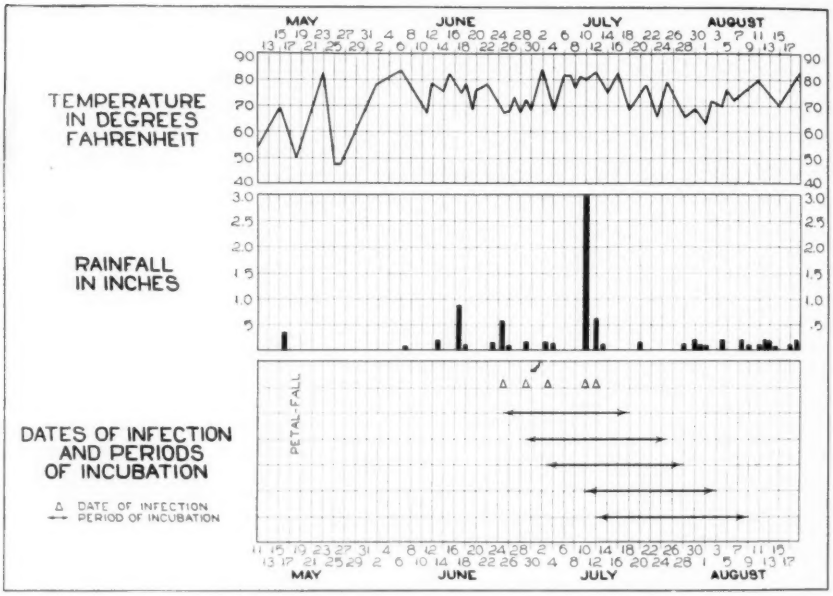

FIG. 3. A graphic summary of certain data relative to blotch-infection experiments and meteorological records at La Fayette, Ind., 1925.

2 or 4 potted trees were placed beneath a diseased tree, removed after the next rain, and replaced in the row of potted trees. During what was considered to be the blotch season, that is, from May to August, potted trees were exposed to infection in this manner throughout each rain. Records were kept of disease development in these trees. The meteorological records for the experiments at La Fayette were secured from the local weather station located at Purdue University and those for the experiments at Mitchell were obtained from the reports of the Paoli station, 12 miles distant. It is realized that the Paoli conditions varied slightly from those at Mitchell, but they constitute the best meteorological records that could feasibly be secured.

The times of natural infection for the years 1925 to 1928, inclusive, at La Fayette and for 1926 to 1928, inclusive, at Mitchell are shown in figures 3–9. Lesions produced on the petioles or midribs were used as an index to infection. Counts of the number of healthy and diseased leaves were made and records kept. The times of first and last occurrences of infection were recorded, and these have been projected in the graphs in figures 1–7. The experiments at Mitchell were at such a distance that the incubation periods could not be determined.

The summer of 1925 at La Fayette was relatively unfavorable to blotch infection (Fig. 3). Petal fall[3] occurred May 19. Infection took place

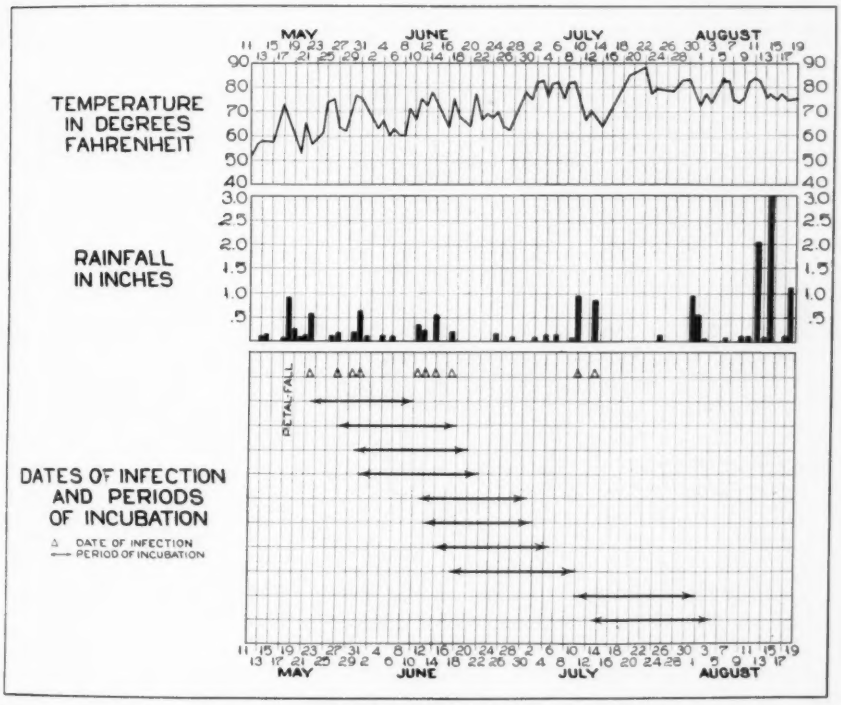

FIG. 4. A graphic summary of certain data relative to blotch-infection experiments and meteorological records at La Fayette, Ind., 1926.

during the 5 rain periods 36, 41, 45, 52, and 54 days, respectively, after petal fall. The protracted dry period early in the season undoubtedly delayed the first infection period considerably, as may be noted by comparison with subsequent seasons. Infection during all rain periods was light.

In the summer of 1926, at La Fayette, there were 10 rain periods during which infection took place (Fig. 4). Petal fall occurred May 19. Infection

[3] The time of petal fall was recorded, when ½ to ¾ of the petals had fallen.

took place during the rain periods, 3, 8, 11, 12, 23, 24, 26, 29, 52, and 55 days, respectively, after petal fall. First infection of this season almost coincides with petal fall, a fact noted also during later seasons, when environmental factors were predisposing to disease production. Data recorded during this summer show that a high humidity prevailed during spring and early summer and that infection occurred during nearly every rain. In late June and early July the humidity was quite low, even with light rains during alternate days. Under these conditions infection did not take place. Infection occurred incident to 1 in. of rain on July 10. There was no heavy infection on any of the potted trees.

The summer of 1926, at Mitchell, 150 miles south of La Fayette, was even less favorable to blotch than at La Fayette (Fig. 5). There were only

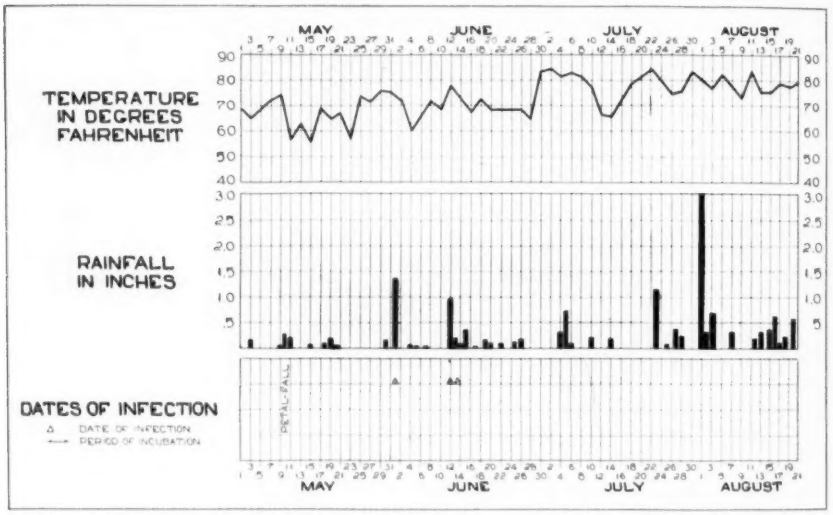

FIG. 5. A graphic summary of certain data relative to blotch-infection experiments and meteorological records at Mitchell, Ind., 1926.

3 rains during which infection occurred and infection was very light. These periods were 21, 32, and 33 days, respectively, after petal fall. Petal fall occurred May 11.

The summer of 1927, at La Fayette, was fairly favorable for blotch (Fig. 6). Rains were frequent, humidity was high, and consequently infection took place frequently. This was a rather early season. Petal fall was completed by May 7 and infection took place during 18 rain periods, 1, 3, 11, 12, 17, 18, 21, 23, 26, 27, 28, 36, 37, 42, 45, 68, 69, and 71 days, respectively, after petal fall. This represents a considerable number of infection periods. The effect of a dry period upon infection during late June may be readily seen inasmuch as no infection occurred during the

first 4 rains following the dry period. The heaviest infection occurred during the 2nd, 4th, 6th, 8th, and 12th rain periods, respectively.

A favorable blotch season was recorded also for the summer of 1927 at Mitchell (Fig. 7). Petal fall occurred April 25, and infection took place during 15 rains, 5, 6, 12, 13, 15, 19, 21, 24, 30, 31, 33, 35, 36, 50, and 58 days, respectively, after petal fall. The heaviest infection occurred during the 5th, 9th, 11th, and 13th rain periods, respectively.

Since the summer of 1928, at La Fayette, was exceedingly moist very heavy blotch infection took place (Fig. 8). Petal fall occurred May 13. There were 25 rain periods during which infection occurred. These occurred 3, 5, 6, 13, 17, 22, 24, 26, 31, 35, 36, 37, 38, 41, 42, 43, 44, 46, 47,

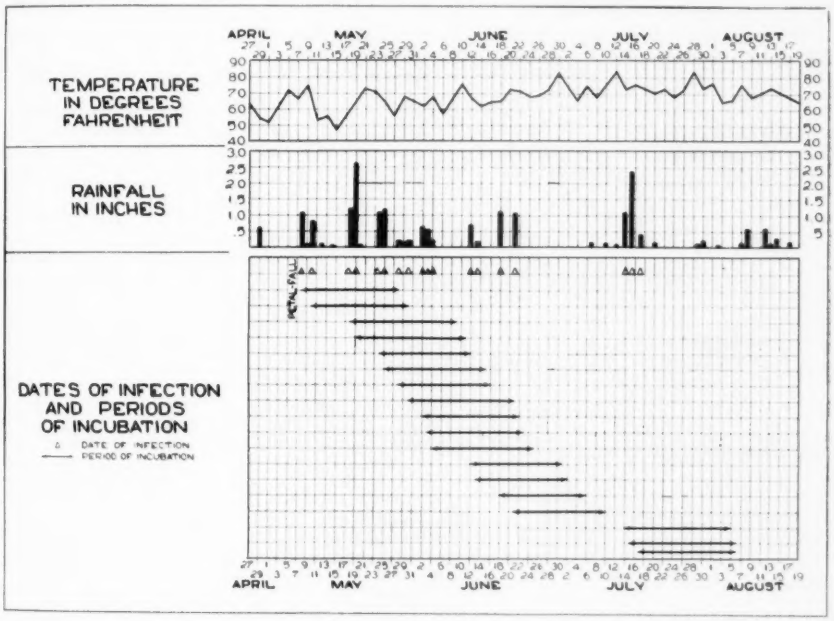

FIG. 6. A graphic summary of certain data relative to blotch-infection experiments and meteorological records at La Fayette, Ind., 1927.

51, 52, 53, 57, 61, and 67 days, respectively, after petal fall. Infection was heavy in all rain periods except the last 2 in which infection was relatively light.

Similarly, a very severe blotch season was encountered at Mitchell in 1928 (Fig. 9). Here petal fall occurred May 9. Infection took place during 22 rain periods, respectively, 5 days before and 8, 9, 11, 17, 18, 21, 26, 27, 28, 30, 31, 35, 36, 40, 42, 43, 46, 57, 61, 65, and 74 days after petal fall. This season, both at La Fayette and Mitchell, illustrates to a marked degree the relation of the abundance of rainfall to the frequency of infec-

tion. The infection that occurred before petal fall was a mere trace. During nearly all subsequent rain periods, infection was very heavy except during the last one. Infection at Mitchell was even heavier than at La Fayette during this season. As many as 43 leaves with petiole lesions were counted on 1 tree, representing 50 per cent of the total number of leaves present.

It may then be concluded that the time of first infection varies considerably, depending on the occurrence of early rains, which may coincide with the normal period when blotch spores are physiologically mature. Usually such a time of maturity immediately follows petal fall, although in one instance its advent preceded petal fall. An examination of figure 3 will show the decided effect of a protracted dry period on earliest infection.

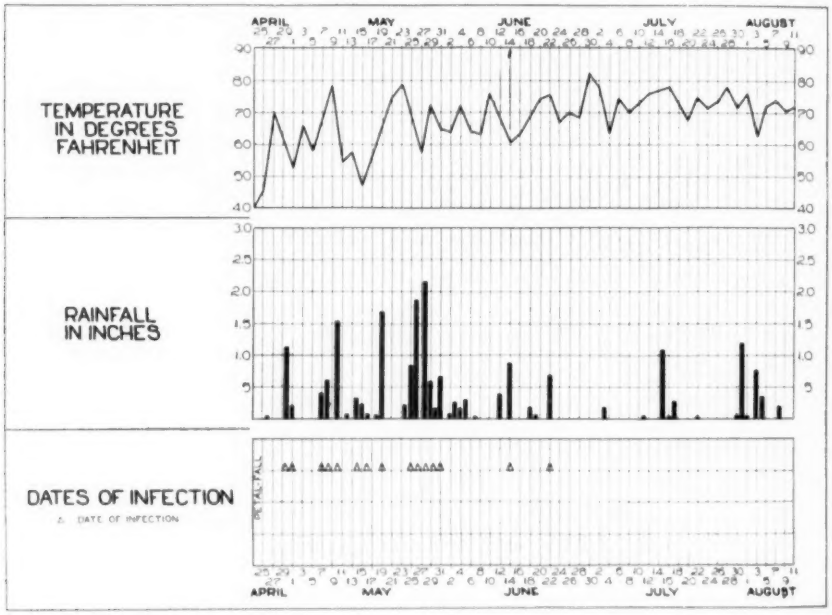

FIG. 7. A graphic summary of certain data relative to blotch-infection experiments and meteorological records at Mitchell, Ind., 1927.

The latest infection usually occurs during early July or the middle of July. Cessation of infection seems not to be dependent alone on lack of moisture but also on another factor. The supply of spores is by no means completely exhausted at this time, since spores continue to be formed in new cankers appearing during May or June. Examination of pycnidia in such cankers in late July and in August revealed abundant spores capable of germination. Since infection occurred no later than the middle of July, it may be assumed that the leaves had become resistant to penetration and infection.

From the data available there is no evidence that temperatures encountered within the usual range of field conditions during the period of natural infection have any sharply limiting influence upon infection. It may be that temperature shortens or lengthens the period of incubation, but no data are available to show that it does. The graphic records for La Fayette (Figs. 3–9) do not show any change in the length of the period of incubation attributable to differences in temperature. It is known from general observations that blotch epidemics are quite severe even during cool seasons. The summer of 1928 was rather cool, as well as moist, and infection was very heavy.

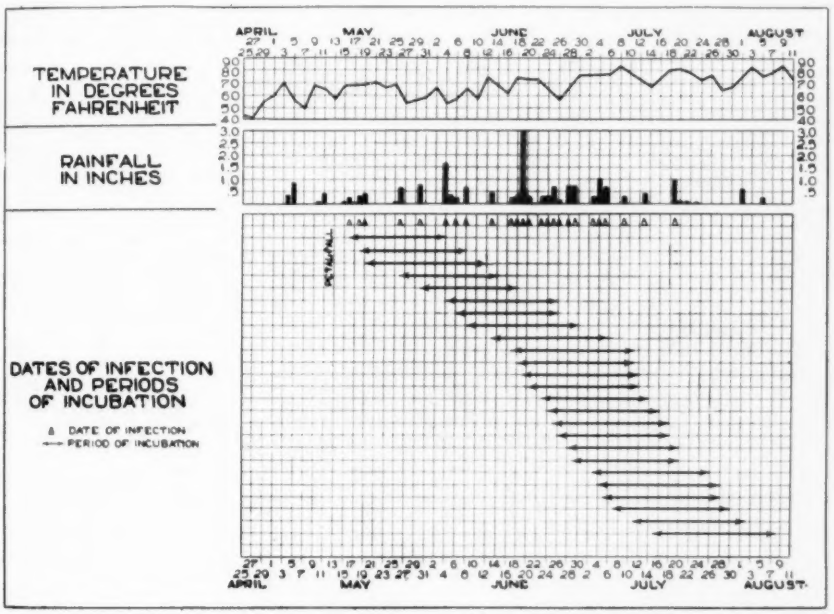

FIG. 8. A graphic summary of certain data relative to blotch-infection experiments and meteorological records at La Fayette, Ind., 1928.

It may be stated, also, that no definite correlation can be made between the occurrence or severity of infection and the amount of precipitation. Observations show, however, that during a period of high humidity a very light rain of approximately 1/10 in. or less may bring about infection, whereas a similar rain following a rather dry period may not be followed by infection. The time interval over which a rain falls undoubtedly plays a large part in bringing about infection.

PERIOD OF INCUBATION

The period of incubation varied from 18 to 24 days, as determined by greenhouse and field experiments at La Fayette. Because of the distance

to Mitchell, the period of incubation could not be determined there. The period of incubation under natural conditions and on trees infected artificially and kept in the greenhouse was about the same. These records are based on petiole lesions, although leaf-blade lesions were also present and usually appeared only a day or two earlier.

CYCLE OF INFECTION

In order to have a better understanding of the cycle of infection of the apple-blotch disease in nature, a large number of counts was made of infected leaves in an experimental block of 60 young Duchess apple trees at Vincennes, Indiana, in which cankers had been excised by M. W. Gardner

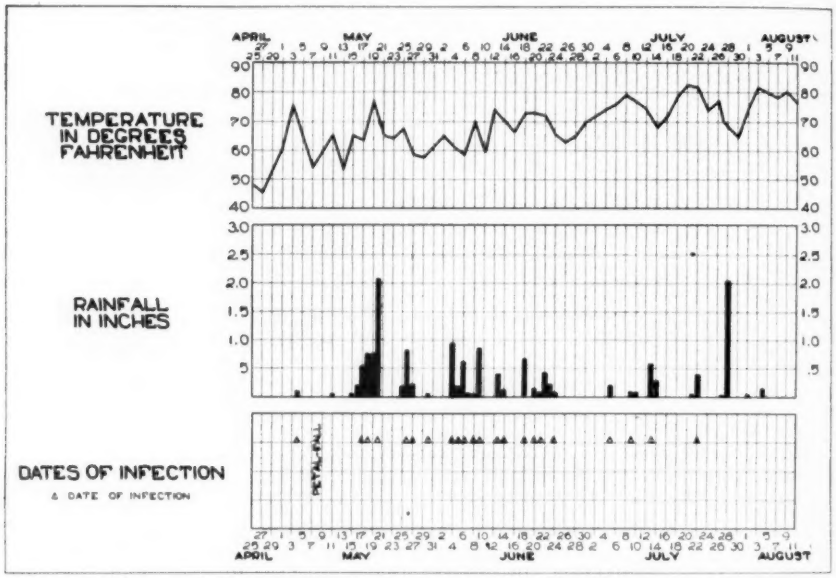

FIG. 9. A graphic summary of certain data relative to blotch-infection experiments and meteorological records at Mitchell, Ind., 1928.

since 1922 and blotch sprays withheld from 1925 to 1927. The counts of petiole infection were made in August, 1927, and the origin of the inoculum was determined by cones of infection that pointed to cankers at the apices. It was found that, in 37 trees that showed infection and in which 12,330 leaves were examined, no infection was traced to cankers on 1926 wood. Infection on 1,615 of these leaves was traced to 72 cankers on 1925 wood and only 2 cankers were found on 1925 wood under which there were no infected leaves. Infection on 35 of these leaves was traced to 6 cankers on 1924 wood. No infection was traced to cankers on 1926 wood nor on 1923 or 1921 wood of which 2 each were found. This is suggestive of a cycle

of infection at least 2 years in duration. However, in fruit counts made in 1928 in the same block of trees by M. W. Gardner, 6 instances of fruit infection were traced to cankers on 1927 wood, indicating an annual cycle of infection.

The field evidence indicates that with an early season the young cankers appearing in May, or possibly a few formed coextensively with petiole lesions the previous season, may be sufficiently well developed by July so that spores are mature and infection may occur. This is usually not the case in northern Indiana, but probably the severe epidemics in the Southern States may be explained by the fact that there is an annual cycle.

It may be seen, then, that in southern Indiana in seasons of moderate rainfall and infection, a 2-year cycle is completed, since spores produced in cankers appearing during the current season do not infect. During consecutive seasons that are moist and conducive to heavy infection and in which early infection occurs the first season and late infection the second, a condition is brought about whereby an annual cycle is effected. The fungus is able to produce spores in time to cause infection the second season; and protracted moist conditions cause a continued development of new leaves of the host, later in the season, that serve as an avenue for infection.

SUMMARY

1. Pycnospores were mature in overwintered cankers in late April at La Fayette and Mitchell, Indiana. In young cankers appearing in May and resulting from infection of the previous season spores were mature in July.

2. Fertile pycnidia were formed on corn-meal agar containing 50 gm. of corn-meal and 25 gm. of agar per liter in about 21 days on thalli containing the original tissue planting. Subcultures did not produce fertile pycnidia even on the same medium.

3. A favorable temperature for fructification was found in the range of 19° to 21° C.

4. A time interval elapsed between morphological and physiological maturity of the spores. Under natural conditions it was about 1½ months.

5. Experiments designed to hasten maturation of spores through the use of various chemicals gave negative results.

6. Spores germinated in water on glass slides formed hyaline germ tubes only. The spores adhered to the slides and were not readily washed away by a stream of water from a faucet.

7. Spores germinated on leaves formed appressoria. Germ tubes and appressoria were olivaceous brown. Appressoria were, on the average, 6 μ wide and 10 μ long. Germ tubes averaged 2 μ wide and 4 to 14 μ long.

8. Young apple trees were successfully infected with spores from natural sources and with spores produced in artificial culture. The fungus was reisolated.

9. Spores produced after the middle of July or late July were of no great importance in the production of diseases at La Fayette and Mitchell, Indiana, probably because the host tissues were no longer susceptible.

10. By exposing in succession for suitable periods healthy potted Duchess apple trees to infection under a diseased tree, experiments during the seasons of 1925 to 1928, inclusive, have shown the time of natural infection to be from late April or early May to about the middle of July. Very early infection occurred when protracted wet weather coincided with the time of physiological maturity of the inoculum, which was commonly found to occur at about the time of petal fall. At LaFayette in 1925, infection occurred 36, 41, 45, 52, and 54 days, respectively, after petal fall. At La Fayette, in 1926, it occurred 3, 8, 11, 12, 23, 24, 26, 29, 52, and 55 days, respectively, after petal fall. At Mitchell, in 1926, it occurred 21, 32, and 33 days, respectively, after petal fall. At La Fayette, in 1927, it occurred 1, 3, 11, 12, 17, 18, 21, 23, 26, 27, 28, 36, 37, 42, 45, 68, 69, and 71 days, respectively, after petal fall. At Mitchell, in 1927, it occurred 5, 6, 12, 13, 15, 19, 21, 24, 30, 31, 33, 35, 36, 50, and 58 days, respectively, after petal fall. At La Fayette, in 1928, it occurred 3, 5, 6, 13, 17, 22, 24, 26, 31, 35, 36, 37, 38, 41, 42, 43, 44, 46, 47, 51, 52, 53, 57, 61, and 67 days, respectively, after petal fall. At Mitchell, in 1928, it occurred 8, 9, 11, 17, 18, 21, 26, 27, 28, 30, 31, 35, 36, 40, 42, 43, 46, 57, 61, 65, and 74 days, respectively, after petal fall.

11. Infection periods were found to be correlated with periods of rain and high humidity.

12. The incubation period is about 18 to 24 days, as determined by the potted-tree experiments.

13. The temperature range encountered in the experimental orchards during the season of natural infection was not found to cause any appreciable change in the length of the incubation period.

14. The fungus was found to be intercellular and, in the petiole and bark was limited to the collenchyma. This was demonstrated by the use of a 1–1000 gold chloride solution following fixation in Gilson's fluid. The host forms cork below the invaded areas. The mycelium in the collenchyma keeps in advance of the newly formed cork.

15. The fungus usually enters the bark of the host by way of the petiole, across the abscission layer, in late July and August.

16. In 1927, at Vincennes, Indiana, infection was traced to cankers found on 1925 wood. In 1928 a small percentage of infection was traced to cankers on 1927 wood. In 1927 the cycle of infection was found to be

at least 2 years in length. In 1928 the cycle of infection was found to be as short as 1 year in length.

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PEANUT WILT IN GEORGIA[1]

J. H. Miller and H. W. Harvey

This investigation was initiated in order to determine the identity of the organisms responsible for a very common wilt of peanuts in Georgia. It involved 3 disease surveys of about 1 week each, 1 beginning June 19, another August 8, and, the last, September 10. Besides the surveys, diseased specimens were sent the writers each week for study.

Most of the peanuts are grown in the southwest corner of the State on very sandy land. Nearly all the marketable peanuts are of the variety White Spanish, while those grown for hog grazing are Georgia or Alabama Runners. The large peanut, or Virginia type, on account of disease, has not been grown successfully.

Last year experiments were conducted in various counties with the Virginia type, with very poor results. Gypsum was used as a dust, as is done in Virginia, but with no evident control. Climatic conditions, being very dry in the early summer and wet in the late season and during harvesting, were very unfavorable. At Ft. Gaines, Virginia Runners yielded 1,036 lbs. per acre; Virginia Bunch, 816 lbs., and White Spanish, 1,305 lbs. This was on sandy land and there was much *Sclerotium rolfsii* Sacc. in the late season. At Cuthbert 1,818 lbs. per acre of Virginia Bunch was harvested. This tract is a red clay, and there was much less disease than on the sandy types of soil.

This summer, conditions were not especially favorable for growth of fungi, but there were more wilt and root rot than last year. Where peanuts had been grown for successive years on the same land the loss was heavy, and it was also considerable on some fields that had not been planted to this crop for 3 or 4 years.

METHODS

Roots or infected areas or stems of diseased plants were washed thoroughly in distilled water, then soaked in mercuric chloride for 5 minutes and placed in Petri dishes in hard agar. Most aerial molds were eliminated in this manner. Pure cultures were then made of all probable pathogens, and these were used to inoculate peanut plants grown in the greenhouse. Supplemental inoculations were made in the field. Positive

[1] The writers wish to express their appreciation to the Tom Huston Peanut Co. for its cooperation in this investigation and especially for the aid rendered by Mr. Bob Barry, Mr. Grady Porter, and the county agents in this district. The disease surveys were accomplished in cooperation with the Division of Mycology and Disease Survey, Bureau of Plant Industry, United States Department of Agriculture.

results were obtained with 3 organisms isolated from a majority of the diseased specimens. These are described below.

1. FUSARIUM MARTII APPEL AND WR. VAR. PHASEOLI BURK.

This organism produces a peanut root rot, which results in the affected plant's first turning yellow, then wilting, followed soon by death. The most severe infections were found in many fields in the region about Blakely and Ft. Gaines. During the June inspection, in one field near Blakely, from 20 to 38 per cent of the plants were either dead or dying. At the Huston Experiment Farm, Ft. Gaines, about 20 per cent of the plants wilted during June. Cotton had preceded peanuts on most farms and no information could be obtained as to root rot or wilt of cotton or other crops planted here in other years.

Hosts. The peanut varieties found to be susceptible were White Spanish, Virginia Runner, Virginia Bunch, Georgia Runner, Alabama Runner, and Namberquarie. The White Spanish was most susceptible, followed by the Virginia forms, and the Namberquarie least, with Alabama Runners fairly resistant. Burkholder (2) describes this variety as causing a dry root rot of several different kinds of beans of the genus Phaseolus, also of black-eye cowpea and of kulti bean. The writers performed no experiments to determine the susceptibility of plants other than the peanut. No previous record has been found of its attacking the peanut.

Description of the disease. The maximum period of infection occurs when the plants are about 2 months old and just blooming. In some cases only 1 or 2 shoots wilt, but in most instances the entire plant succumbs. Because of the drought at this period, there were very few lateral roots or adventitious roots or peduncles in the ground; so the entire water supply came through the tap root. On examining the roots, necrotic areas were found in the upper part, just below the crown (Fig. 1, A). The root at this point was completely rotted, became brittle, and broke off very easily.

The first signs of the disease consist in a yellowing of the upper leaves, and during the drought this condition was followed quickly by wilting and death of the plant. There are no external signs to suggest a Fusarium as the causal agent. Determinations can be made only by laboratory study.

Very young lesions begin as small, elongate spots, slightly sunken, with light centers and dark brown borders. As the lesion ages, the root is girdled, the spot increases to 1 to 2 cm. in length, the epidermis and cortical tissue become shredded, and the root dies. Cross and longitudinal sections were made through the necrotic area. In the disintegrated cortical region and also in the xylem, fine hyphae were found. The epidermis, cortex, phloem, cambium, and xylem rays are completely destroyed. The walls of xylem vessels are darkened. These anatomical changes extend up for 2 or more

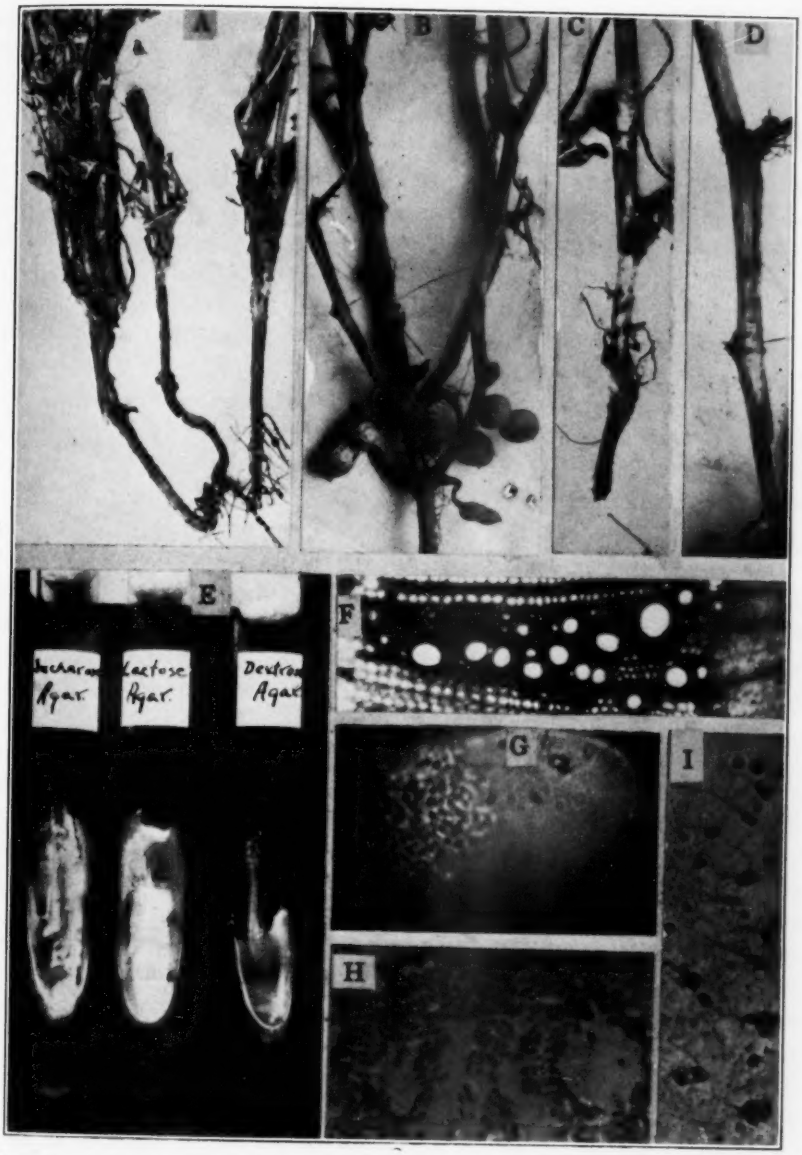

FIG. 1. A. White Spanish plant gathered June 26. The rotted roots in culture produced the organism *Fusarium martii* var. *phaseoli*. B. White Spanish, collected August 10, Huston Experiment Station, showing bacterial lesions on lower parts of shanks. C. The same showing lesions higher up on stems. D. Bacterial lesions enlarged showing characteristic shredded appearance when old. E. Agar slants of *Bacterium solanacearum*, 8 days old. F. Cross-section of stem (×195), showing tracheal tubes plugged with bacteria. This is to be seen in the very black areas. G. A single vessel showing bacteria. ×1090. H. Bacteria from pure culture. I. Bacteria showing polar flagella.

centimeters beyond the visible lesion. There is no plugging of xylem vessels with hyphae, as Atkinson (1, Fig. 6) described for *Fusarium vasinfectum* in cotton roots.

Wilted plants were observed scattered all over the fields, with no appearance of central focal points. There was not much evidence to make one suspect that the fungus was spreading from one plant to another.

Etiology. The pathogen, *Fusarium martii* var. *phaseoli* was determined as such by Sherbakoff. In litt. Aug. 8, 1931, Sherbakoff states that he considers Burkholder's organism to be identical morphologically with his *F. martii* var. *minus* Sherb. He further says that the variety *phaseoli* morphologically is about the same as the species *martii* but differs from it in causing a dry root rot of beans.

Although Burkholder (2, p. 1,024, tab. 3) has proved the causal relation of his organism with root rot of bean and while this organism resembles it morphologically, no experiments have been made to prove them physiologically identical.

Inoculation experiments. Sixty plants were grown in pots in the laboratory. Selected seeds were hulled, then soaked for 5 minutes in mercuric chloride (1:1000), washed in distilled water, and planted in pots containing sandy loam. The soil and pots were sterilized for 1 hour at 20 lbs. pressure. After these plants had grown about 6 in. high 20 were inoculated with the Fusarium conidia by puncturing the epidermis just below the crown and 20 were inoculated by pouring a spore suspension on the soil. In 10 of the latter some roots were broken, and in 10 they were not broken. Twenty pots were left as controls. In all cases where abrasions had been made on roots the plants wilted within 5 days, but negative results were obtained where the epidermis was not first ruptured. The wilted plants were examined, and the Fusarium was found growing on decayed roots.

An inoculation experiment was then made to determine the toxicity of a Fusarium filtrate. Ten flasks containing Richard's solution made up according to Fahmy (3, p. 545), differing from the standard in containing 1,500 cc. of water instead of 1,000 cc., were inoculated with the fungus, and a copious growth was obtained within 15 days. Then each was filtered under sterile conditions, and young plants were placed in 6 flasks along with parts of mature plants. These wilted within 3 days (Fig. 2, A). Similar plants placed in the solution without the fungus did not wilt at end of 15 days.

Field inoculations on mature plants were made on August 10 at Ft. Gaines. These plants were examined September 10 and no wilt was produced. The Fusarium was reisolated from a few decayed roots and peduncles but no serious disease was produced on these mature plants.

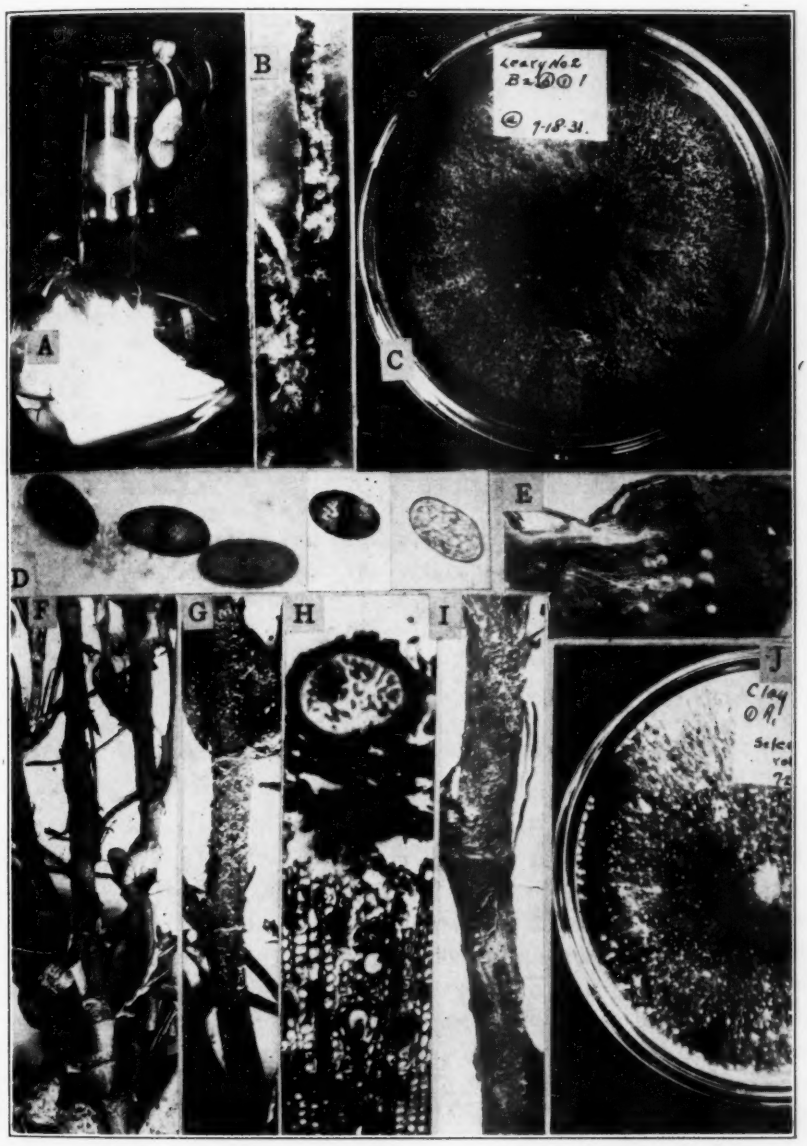

FIG. 2. A. Wilted plant after 48 hours in filtrate from *Fusarium martii* var. *phaseoli*, growing on Richard's solution. B. Fusarium developing on affected crown and root in dextrose bean agar. C. Pure culture of *Fusarium martii* var. *phaseoli*, 40 days old. D. Conidia of *Diplodia natalensis*. ×655. E. Sclerotia on roots and soil. ×9. F. White Spanish plant showing Diplodia pycnidia covering lower branches. G. Part of F enlarged by 10. H. Cross section of plant showing Diplodia pycnidium and disrupted cortex, phloem, and cambium. ×195. I. Lower part of stem, ×9, of White Spanish, August 10, showing mycelium of *Sclerotium rolfsii*. J. Pure culture of *Sclerotium rolfsii* from Huston Experiment Station.

Description of fungus. The following characters were brought out by growing this fungus on the various media described below.

Microconidia on aerial mycelium typically present, mostly 0-septate, 2.8–14 × 3.5–5.6 μ, 1-septate 14–22.4 × 4.2–5.6 μ, oval to oblong, occasionally 1-septate spores constricted at septum; macroconidia mostly 3-septate, of nearly uniform diameter throughout, usually slightly broader toward base, rounded apices or slightly constricted, 24.4–39.2 × 4.2–6.3 μ. Aerial mycelium white drab; substrate on neutral agars brown to reddish brown or reddish orange on rice; chlamydospores single or in chains, verrucose when mature, 8–12 μ in diameter, globose, intercalary or terminal; conidia mostly borne in false balls at apex of long conidiophores (Figs. 2, A–C and 3).

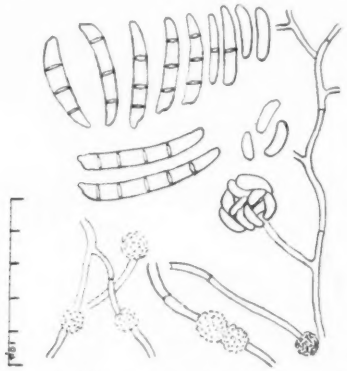

FIG. 3. *Fusarium martii* var. *phaseoli* showing micro- and macrospores and chlamydospore formation.

Cultural characters. Parts of roots with lesions were cut off and sterilized for 5 minutes in mercuric chloride (1:1000) and plated in Petri dishes in hard, bean agar. This was done with all specimens collected in the June inspection and, from then on, from plants sent in from this region. The Fusarium appeared on 80 per cent of such lesions within from 2 to 3 days. It grew very slowly on such stems, producing small sporodochia, such as are seen in figure 2, B. These were grayish white in color. After 4 or 5 days these cultures were overrun with aerial molds. Pure cultures were obtained by lifting off single spores, or a false ball of spores, from a single conidiophore and placing them on sterile media.

Much aerial mycelium with 85 to 90 per cent of microspores was obtained on rice and peanut agar and on whole wheat grains and broken steamed peanuts. Chlamydospores appeared on the rice and peanut agar but not on the other 2 media. The color change was most pronounced on the rice agar.

Very little aerial mycelium was produced on potato-dextrose agar, wheat agar, bean-sucrose agar, and plain potato agar. The spore types on these

media were 80–90 per cent 3-septate macrospores with a very small percentage of 4- and 5-septate spores and very few microspores. Small pseudopionnotes of macrospores formed on all of these. Also, few chlamydospores developed.

On sterile agar very few conidia formed from scant aerial mycelium, but chlamydospores developed abundantly.

Effect of weather conditions and age of plant. This fungus produces most serious damage to young plants during hot dry weather. These facts are shown by the inoculation experiments in which very young plants in pots wilted within a few days; and, although mature ones in the field 10 days after inoculation showed some evidence of root rot, it was not sufficient to damage seriously the production of peanuts. In the field the effect was worse during the extremely hot days of June and early July, when the plants were young and the root systems poorly developed. After the rains began in middle July the damage from Fusarium was negligible. Burkholder (2, p. 1021) says: "The effect of the fungus is rather to kill and dry up the tissue as it progresses up the root, thus greatly reducing the root system of the plant. The roots that remain healthy or are able to carry on their normal function are the surface roots." "If a prolonged dry spell occurs at the time of blossoming and pod production, it is very injurious to the diseased plants. The yield, without doubt, will be reduced over fifty per cent." These statements apply very well to the peanut wilt.

2. BACTERIUM SOLANACEARUM E. F. SM.

Most of the plants affected with dry root rot also contained bacterial lesions on the lower parts of stems. As the summer advanced less Fusarium was observed, and, beginning with the middle of July, the majority of diseased peanut plants contained this organism.

The distribution of this bacterial wilt in Georgia was more wide-spread than that noted for *Fusarium martii* var. *phaseoli*. At first it was seen chiefly in Clay and Early counties, but later in the season specimens from all the peanut-growing counties exhibited this disease.

A wilt caused by this organism has been noted before. Van Breda de Haan (5, p. 151) describes from Java a type of wilt that seems to be identical with that found in Georgia. The xylem vessels are blocked with bacteria and the plants turn yellow and die. Fulton and Winston (4, p. 43) report a similar condition in North Carolina.

Hosts. The varieties White Spanish, Virginia Bunch, Virginia Runner, Alabama Runner, Georgia Runner, and Nambyquarie were all infected, but the White Spanish appeared to be most seriously damaged and the Nambyquarie least. All of the above Runner types were attacked but with much less wilt than the Bunch types.

Besides the peanut many other plants are susceptible. Stevens (8, p. 36) mentions Datura, *Solanum nigrum*, Physalis, Petunia, Nasturtium, bean, pea, Ricinus, vanilla, Helianthus, Dahlia, Cosmos, tomato, tobacco, and potato. As the cause of the Granville wilt of tobacco it is very common in parts of North Carolina and occurs occasionally in Georgia.

Hartley (6) reports that tests were conducted in Java to determine susceptibility of various varieties of the peanut to *Bacterium solanacearum* and that American varieties, especially the Valencia, proved more susceptible than the Javanese varieties at Buitenzorg. The Tjina variety was most resistant. All plants on contaminated soils became infected.

Description of the disease. The term "wilt" does not apply to all the symptoms exhibited by plants affected by this bacterium. Only young plants and an occasional branch on a mature plant actually wilt. The term "yellows" is more expressive of the symptoms shown by mature plants.

The diseased plants at first turn yellow, and, if the attack be severe, this condition is followed by death of the entire plant or of single branches. When the single branch died death was found traceable to bacterial lesions on the stem, such as is seen in figure 1, B, C, and D.

The field signs of the disease are to be seen in the wilting of leaves and stems after they have turned yellow. The bacterial wilting can be determined by the dark brown spots, visible to the eye in cross-section, in the xylem and pith. This character serves to distinguish it from the root rot caused by Fusarium. The latter does not enter the xylem appreciably except in extreme cases of rotted roots.

The lesions begin as small black spots, which spread rapidly in a radial direction. A large lesion (Fig. 1, D) shows a light slightly sunken area surrounded by a dark margin. The central part is composed of shredded epidermis and cortical tissues. This splitting is typical of an old bacterial infection.

Histological changes. Infected parts were fixed and killed in Flemming's Weak, Flemming's Strong, and Carnoy Solutions, embedded in paraffin and sectioned. The young plants cut very well, but older ones containing much lignified tissue have to be sectioned from celloidon. Sections were cut rather thick (10, 15 and 20 μ) in order to hold the bacteria. These were stained with anilin-fuchsin and iron-alum-haematoxylin, counterstained with safranin. The latter combination gave the best results.

The bacteria penetrate either through an insect wound or directly through lenticels, spreading laterally as well as towards the center. Epidermis, cortex, phloem, and cambium are killed and cell walls become stained brown. Xylem vessels become packed with bacteria, and living xylem rays are destroyed. (Fig. 1, F.) In this stem cross section the

darkened areas surrounding empty vessels are cells that have become entirely occluded with bacteria. In figure 1, G is seen a single vessel in cross-section showing bacteria. In the pith brown areas are filled with bacteria.

Etiology. This organism has been identified as a causal pathogen of peanut wilt by van Breda de Haan (5) and by Fulton and Winston (4). Proof of this is shown again by the following experiments.

Inoculation experiments. Several hundred plants were grown in sterile soil from seed treated as explained before. When the plants were about 6 in. high they were divided into 3 lots; 1 was inoculated by rupturing the surface with a needle containing active bacteria, another by pouring a suspension of bacteria in distilled water on the soil, and the last lot was kept for a check.

At the end of 8 days all plants appeared to be approximately even, though the inoculated ones were slightly yellowed. Some of these were pulled up and examined. Where the soil had been inoculated the tap roots were black and shrivelled and almost dead, but many surface roots appeared to be keeping the plants alive. Cross-sections above the darkened areas showed bacteria in pith and xylem, with characteristic browning, even above the crown. In the stem-inoculated plants the lesions did not increase much in size, but bacteria were found in xylem and pith. Most of these died within 3 weeks.

Field inoculations were made on mature plants at Ft. Gaines and at Athens on August 10 and 21, respectively. Stems were inoculated, and dilutions were poured on the soil at the bases. Examinations were made on August 29, with no macroscopic results, but such stems, when sectioned, showed bacteria in xylem vessels. On September 10 all inoculated plants had become distinctly yellow in contrast to the green of the noninoculated ones.

Effect of weather and age of plant on bacterial growth. High temperatures are more favorable to the growth of the organism, and this accounts for the wide-spread prevalence in the Southern States and its nonoccurrence very far north. It grows more rapidly in young plants during very hot weather. The attack was greatest in June and July. For successful inoculations the writers found that bacteria had to be taken from 2- to 3-day-old cultures and placed in young plants. Such plants, very succulent, were more conducive to rapid growth and spread of the organism. In mature plants bacteria filled up some of the vessels in the xylem and appeared to become quiescent quickly, and not enough of such vessels were occluded to cause death. However, occlusion produced a slight yellowing of leaves, and such plants were more rapidly attacked by such partial parasites as *Diplodia natalensis* Evans.

Control. At the Huston Experiment Farm both sulphur and copper sulphate were used in a control experiment. These fungicides were placed in the ground when the peanuts were planted and were also dusted on plants about a month old. Only negative results were obtained.

This is definitely a soil organism, and, so far, the only means of control advanced are to be found in a long rotation. The important point here is to know what plants are susceptible, including weeds, and to keep these off the ground for a period of at least 5 years, according to Stevens (9, pp. 26 and 257). Wolf and Stanford (11, p. 164) add to this list many weeds common in cotton fields, so that clean cultivation should be practiced with a resistant crop.

No work has been done in breeding resistant varieties of peanuts in this country, but such has been done in Java, and it is possible that resistant Javanese varieties could be established here.

3. DIPLODIA NATALENSIS EVANS

This fungus was found in the late summer in fields that had been severely infected with bacterial wilt. It appeared to be causing a foot rot on from 10 to 20 per cent of plants in 1 field in Clay County. Many plants, still green and healthy in the tops, were covered with pycnidia at the base.

Cultures were made from single spores obtained from pycnidia on peanut stems. Inoculations were then made from the resulting mycelium and also from spores on plants grown under control conditions and on many plants in the field. These attempts all uniformly failed to produce infection.

All plants containing the Diplodia in the field also had *Bacterium solanacearum* in the vascular system as well as bacterial lesions on the stems. The evidence seems to justify one in concluding that this species of Diplodia is a weak parasite following attacks of more virulent organisms.

The position of the pycnidium in the decayed cortical tissue is shown in figure 2, F, G, and H.

4. SCLEROTIUM ROLFSII SACC.

This organism is wide-spread over the entire peanut belt, having been isolated from specimens from almost every county. There has been no special epidemic in Georgia, but it was particularly severe in isolated fields. The greatest losses were in light sandy lands near Ft. Gaines. In one 60-acre field the yield was reduced to about 600 lbs. per acre, which is a 50 per cent decrease of an estimated yield in July. At the Huston farm last year the yield of White Spanish was 1,305 lbs. per acre with much *Sclerotium rolfsii*, and this year, on the same land, that yield was cut to about 300 lbs. per acre, due chiefly to this organism and the bacterial wilt.

This fungus attacks living plants, and, from data gathered the last 2 years, it is worse during the late part of the season, especially if there is much rain.

During the month of August about 25 to 75 per cent of plants sent in from Clay County had this infection. Because of a very dry fall not many plants died, but the injury to roots and peanuts was considerable.

This disease is not a new one on peanuts in the Southern States, although it appears to have been overlooked as an important one in Georgia. Wolf (10, p. 143) says: "This disease appears to confine its attacks to the roots and peas. Usually there is no indication of the disease in the appearance of the aboveground parts. When, however, the plants are dug a greater or less proportion of the peas will be found to have decayed." Besides this citation from Alabama, McClintock (7, p. 441) found it causing a wilt in the peanut plots of the Virginia Agricultural Experiment Station.

Hosts. This organism is known to produce wilt, blight, or root rot on a great many hosts. Wolf (10, p. 147) found many legumes susceptible, but the Brabham and Iron cowpea escaped attack. McClintock (7, p. 448) says: "Tests of six varieties of peanuts and one hog goober, *Worandzia subterranea*, planted in soil badly infested with *S. Rolfsii* showed that the Valencia peanut is most susceptible to this organism and that Spanish, Tennessee Red, and Virginia Bunch are respectively resistant in the order named, while the Virginia Runner, African, and Hob Goober are practically immune to *S. Rolfsii*."

Description of the disease. This summer the writers found that most of the trouble would come under the heads of root rot and rot of lower shanks and a great deal of rot of peanuts rather than wilt. There was very little actual wilting of entire plants. In most cases only single branches died, and when such plants were pulled up the fungus was found covering roots and peanuts. During the short rainy season in midsummer affected plants contained a thick envelope of white mycelium on lower shanks with many developed sclerotia, but in the following drought the fungus was confined to parts below the ground and the growth of mycelium was much reduced. All parts covered with this mycelium turn brown and decay rapidly.

No attempts were made to prove the parasitism of this organism, as that has been done by McClintock (7) and Wolf (10).

Control. There are no known methods of soil treatment that successfully control this disease in the field. Experiments with soil disinfectants at Ft. Gaines were unsuccessful. Also, the great number of susceptible plants makes it impracticable to attempt to eradicate it by crop rotation. The only hope lies in planting resistant varieties of peanut in sandy lands

known to be infested. This fungus rarely becomes a serious problem on heavy land.

The writers have found the Alabama Runners least susceptible of the varieties grown in Georgia. Virginia Runners are also fairly resistant, but the pods are so large and they mature so late that even a small amount of infection on the lower parts of stems will prevent many of the pods from filling out.

OTHER ORGANISMS REPORTED AS PRODUCING ROOT ROT OR WILT

Fusarium vasinfectum Atk. was isolated only once from a diseased root collected near Blakely. It was not considered as a cause of any of the disease this year, and no experiments were conducted with it.

Phoma sp. was reported by Wolf (10, p. 128), as causing a stem rot at Auburn, Alabama. This was not found during this investigation.

Phymatotrichum omnivorum (Shear) Dug. was not seen.

SUMMARY

1. *Fusarium martii* var *phaseoli* is the causal agent of an early, or seedling wilt. This depleted the stand 10 to 38 per cent in Clay and Early counties in 1931. The injury is very slight on mature plants.

2. *Bacterium solanacearum* is the cause of an early wilt followed by stem- and root-rot-producing yellows in mature plants. Such plants are badly attacked by *Diplodia natalensis* or *Sclerotium rolfsii*. Bacterial lesions, with the plugging of xylem vessels in those regions, on lower stems and peduncles, prevented many of the larger types from filling out the pods.

4. *Sclerotium rolfsii* produces a serious root and peanut rot. It was most serious in fields that had much early wilt. This fungus, in 1931, developed most abundantly in August on sandy lands that were so infested last year.

5. Control appears to lie in varieties resistant to *Bact. solanacearum* and *S. rolfsii*.

6. The Alabama Runner is most resistant of the varieties adapted to Georgia and the White Spanish is most susceptible.

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OBSERVATIONS ON THE PARASITIC ABILITY OF CERTAIN SPECIES OF PYTHIUM

F. K. Sparrow, Jr.

In the course of a morphological study conducted several years ago on certain species of Pythium the writer became interested in determining whether or not these fungi, all found in nature as parasites of algae, could attack other algae as well or even infect such very dissimilar hosts as flowering plants.

A few experiments of a rather preliminary nature were performed, the hope being that these would be supplemented in the future by a more exhaustive study. While neither the materials dealt with nor the data obtained were extensive, yet it has seemed desirable at this time to give a brief account of the results, even though they may be somewhat fragmentary.

MATERIALS AND METHODS

The 3 species of Pythium, *P. dictyosporum* Racib., *P. angustatum* Sparrow and *P. adhaerens* Spar. (5, 6), were all obtained from infected green algae, collected in the vicinity of Cambridge, Massachusetts, in 1926–27. *Pythium adhaerens* was found parasitic in *Rhizoclonium hieroglyphicum* Kütz., whereas the other 2 were isolated from *Spirogyra crassa* Kütz. Details of the method of isolation are given in the 2 previously cited papers by the writer.

The methods of fruit inoculation were similar to those outlined by Drechsler (2) and Harter and Whitney (4). Inoculation experiments designed to test the capacity of the fungi to produce root rot followed the method proposed by Miss Buisman (1), while Drechsler's technique was used in the damping-off work (3). In the latter instance eight 7-in. pots of sterilized soil were used for each of the plants (sugar beets and radishes). In each series 4 pots were inoculated with the fungus growing in Petri dishes of corn-meal agar and 4 were inoculated with corn-meal agar alone (controls). One hundred seed balls of sugar beets were planted in each of the pots, while, in the case of the radishes, a medium density of seeds was sown. By frequent watering a high degree of moisture content in the soil was maintained.

The technique employed in the inoculation of the algae was as follows: Twenty hanging-drop cultures (van Tieghem cells) were prepared, each of which contained a number of individuals of the alga in a large drop of sterilized water taken from the pond in which the organism was found. Before the cover glasses were sealed on, the algae in each of these cultures were carefully examined for filamentous fungi and the possible presence

of free zoospores. If such were found, the van Tieghem cells containing them were discarded. Ten cultures were inoculated by placing in the drop a bit of washed mycelium of the particular fungus taken from a pure culture grown in a liquid nutrient medium. The other 10 cells were not inoculated. After sealing the cover glasses, the cultures, both inoculated ones and controls, were either immersed in the same region of the pool from which the alga had been collected, or, if this procedure were not practical, were left under conditions of light and temperature that closely simulated the natural habitat.

In some instances balanced cultures of the alga contained in small jars were similarly inoculated and suitable controls maintained. However, the difficulties encountered in following the course of the infection and especially the detection of the infection, as well as the short time during which data were taken, made this method impractical. It was interesting to note, however, that the results were in entire accord with those obtained by using the hanging-drop cultures.

While the use of hanging-drop cultures would seem at first like placing the algae under very restricted and unnatural conditions, the time during which observations were made was so short, usually from 6 to 10 hours, that it is highly improbable that any marked lowering of the resistance of the organisms occurred. In fact, not only did the algae fail to show signs of lowered vitality but, on the contrary, cells in an active state of division were nearly always observed, even after several days in these cultures.

RESULTS AND DISCUSSION

The results of these experiments to determine the pathogenicity of the 3 fungi to various algae are presented in the following table.

The foregoing table discloses several interesting facts. None of the fungi, under the conditions of the experiments, was found to possess the ability to infect any large percentage of the various algae used. *Pythium adhaerens* was the most virulent of the 3, infecting a member of the Myxophyceae, 3 members of the Chlorophyceae, and one of the Chrysophyceae. It is interesting to note that the gelatinous sheath, often exceedingly thick, as in Microcoleus, Nostoc, and Batrachospermum, offered no resistance to the incursions of the 3 fungi, although the latter were unable to penetrate the cell wall. The ease with which *P. adhaerens* and *P. dictyosporum* infected Tolypothrix was rather unusual, in view of the fact that no members of the Oomycetes, with the exception of Resticularia, have been reported on any of the Myxophyceae.

In the following table are summarized the results of the inoculation of various fruits as well as the results of the root-rot experiments.

TABLE 1.—*Results of inoculation of various algae with Pythium adhaerens, P. dictysporum, and P. angustatum*[a]

Host	Inoculum					
	P. adhaerens		*P. dictyosporum*		*P. angustatum*	
	Inoculated	Control	Inoculated	Control	Inoculated	Control
Nostoc sp. (?)	–	–	–	–	–	–
Oscillatoria sp. (?)	–	–	–	–	–	–
Tolypothrix sp. (?)	+	–	+	–	–	–
Microcoleus sp. (?)	–	–	–	–	–	–
Rhizoclonium hieroglyphicum	+[b]		+	–	+	–
Spirogyra crassa	+	–	+[b]		+[b]	
Ulothrix zonata Kütz.	+	–	–	–	–	–
Vaucheria sessilis DC.	–	–	–	–	–	–
Oedogonium sp. (?)	–	–	–	–	+	–
Closterium acerosum	–	–	–	–	–	–
Nitella flexilis	–	–	–	–	–	–
Synedra sp. (?)	+	–	–	–	–	–
Batrachospermum moniliforme Roth	–	–	–	–	–	–

[a] A plus sign denotes that infection of algae occurred in all of the inoculated hanging-drop cultures; a minus sign indicates that no infection occurred.
[b] The host on which the fungus was found in nature.

From an examination of table 2 it is evident that in the majority of cases the fungi are incapable of living parasitically on the fruits used, at least so under the conditions of the experiments. In 8 instances, however, complete destruction of the inoculated fruits resulted. Five of these cases, which included representatives of the Solanaceae and Cucurbitaceae, were producted by *Pythium adhaerens*, the species also found to be most successful in parasitizing various algae. The 3 varieties of cucumbers were the most susceptible of all the fruits used, being attacked by both *P. adhaerens* and *P. dictyosporum*. The rot produced by the fungi on the various fruits was of a "leak" type, a watery exudation accompanying the disintegration of the tissue.

With respect to the root-rot experiments, it may be noted that the varieties of the 2 plants used, under the conditions of the experiment, were susceptible to *Pythium adhaerens*. In addition, Mammoth White Cory

TABLE 2.—*Results of fruit-rot and root-rot experiments*

Host	Inoculum					
	P. adhaerens		*P. dictyosporum*		*P. angustatum*	
	Inoculated	Infected	Inoculated	Infected	Inoculated	Infected
Virulence to fruit[a]						
Purple plums	6	0	6	0	6	0
Snap beans	30	0	30	0	30	0
Tomato (green and ripe)	14	14	12	0	12	0
Eggplant	4[b]	0	2	0	2	0
Green pepper	4	0	4	0	4	0
Cucumber						
Granite State	12	12	12	12	6	0
White Spine	12	12	12	12	6	0
White Wonder	8	8	6	6	4	0
White Summer Crookneck squash	4	0	4	0	2	0
Yellow Summer Crookneck squash	4	4	4	0	2	0
Des Moines squash	4	0	4	0	2	0
Pattypan squash	4	0	4	0	2	0
Ability to produce root rot[c]						
Corn (Mammoth White Cory)	21	15	12	0	12	5
Pea (Breck's Old Glory)	21	10	12	0	12	0

[a] In all cases of fruit rot the same number of controls were used as the number inoculated. No cases of infection by a Phycomycete were observed.

[b] All fruits finally became invaded by various *fungi imperfecti*. Careful examination showed no phycomycetous mycelium in the infected parts.

[c] Nine control plants were used in each instance, all of which were in a healthy, vigorous condition at the termination of the experiments.

corn was parasitized by *P. angustatum*. *Pythium dictyosporum*, however, was unable to affect the roots of either peas or corn. The infected roots were easily recognized externally by their glassy brown aspect.

It may be justly argued that, inasmuch as pea and corn plants are not aquatic, they were cultivated under circumstances unfavorable for them but quite favorable for the fungi. This question has been considered by Miss Buisman (1) with whom the present writer is in agreement. She says, in part, "Phycomycetes . . . are water-fungi and easily produce swarmspores in mineral solutions, so that in water-cultures the circumstances are very favorable for infection. The pea seedlings, however, grow so well in v. d. Crone's culture solution, and produce such a healthy root system, that according to my opinion, they may well be compared with plants grown

under normal circumstances. At the utmost one may get an exaggerated idea of the pathogenicity of the parasite . . .".

The results of the attempts to produce damping-off in sugar beets and radishes by inoculation with the 3 species of Pythium are summarized in the following table.

TABLE 3.—*Percentage of infection resulting from artificial inoculation of sugar beet and radish with Pythium adhaerens, P. dictyosporum, and P. angustatum*[a]

Host	Inoculum					
	P. adhaerens		*P. dictyosporum*		*P. angustatum*	
	Inoculated	Control	Inoculated	Control	Inoculated	Control
	Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
Sugar beets[b] (Klein's Wanzleben)	85	0	0	0	0	0
Radish (Rice's Early Scarlet Globe)	0	0	0	0	0	0

[a] Expressed in a percentage of Pythium-infected seedlings of the total number of seedlings. Those infected by *Phoma betae* were *not* counted.

[b] In all cases a very small percentage of the plants were attacked by *Phoma betae*.

It may be seen from the foregoing table that of the 3 fungi only *Pythium adhaerens* was capable of producing damping-off, and that, only of sugar beets. There was no question of the pathogenicity of the fungus to this plant for every one of the damped-off seedlings contained not only mycelium but numerous oospores of the parasite. The development of the asexual stage of this fungus was easily induced by immersing infected seedlings in water for a few days. In view of its virulence to this host, it seems probable that, upon further investigation, *P. adhaerens* will be found capable of producing dampingoff in other hosts.

In conclusion, the writer wishes to reiterate that these few experiments cannot be considered as final but are rather of a preliminary nature. For example, with respect to the infection experiments on algae, it is quite possible that varying environmental conditions may make the alga more susceptible to the fungus at one time than at another. It is hoped that an opportunity will be afforded in the future to make a more exhaustive and more precise study of the pathogenicity of these fungi.

The writer wishes to express his thanks to Dr. F. A. Guba of the Massachusetts Agricultural College Experiment Station, at Waverly, for

his many courtesies, to the Director of the Biological Laboratory, Long Island Biological Station, for the use of the facilities of that institution, and especially to Prof. W. H. Weston, Jr., under whose guidance this work was done.

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PHYTOPATHOLOGICAL NOTE

A correction.—In the paper entitled "Immunity in Plants" (PHYTOPATHOLOGY **22**: 95–102. 1932) the following statement on page 97: "In fact, Hursh, among others, in a study of a large number of wheat plants, observed that a parallelism exists between resistance and acidity as expressed in terms of pH (13)" should read instead: ". . . in a study of wheat plants, observed that no parallelism exists between . . ."—J. A. DE TOMASI.

BOOK REVIEW

Hubert, Ernest E. *An Outline of Forest Pathology.* v – viii + 543 pp., 168 figs. John Wiley and Sons, Inc., New York; Chapman and Hall, Limited, London, 1931. Price, $6.00.

Professor Hubert's textbook of forest pathology is the first of its kind produced in America. Fortunately for the undertaking, the author is well equipped by breadth of experience in research and knowledge of forestry to perform this much-needed service. The work is predominantly American in the sense that it deals particularly with the materials and the pathological problems of the forests and the forest products of the United States and Canada. Many results of the observations and the investigations of the pioneers in American forest pathology, supplemented by applicable European data, are now made conveniently accessible and the sources of information are supplied by copious references to the literature.

The book is divided into 3 main sections, entitled: Part I. Introduction (pp. 1–106); Part II. Forest Pathology (pp. 107–445); Part III. Wood Pathology (pp. 446–531).

Part I consists of 7 chapters. Chapter I is headed "Historical"; aside from a brief reference to earlier studies on decay in wood, it consists mostly of a list of some of the forest pathologists, past and present, and a brief outline of the organization of forest-pathology services in the United States. Chapter II, The Trend in Forest Pathology, is more correctly a brief, incomplete, historical sketch than a clear exposition of the author's thesis. Chapter III, entitled the Classification of Tree Diseases, is prefaced by a definition of forest pathology "as the science of tree diseases, their prevention and control," a definition that is not satisfying. It is too broad in that it does not exclude orchard and ornamental trees and too casual in that it fails to mention the forest as the central interest, often the sole unit in prophylaxis. The author more accurately reveals his real conception of the subject in Chapter V, The Relative Importance of "Tree" Diseases, where, in passing, he criticizes mere interest in individual trees rather than "carefully planned and premeditated study of a large group of trees or of a forest." The table presented at the close of the third chapter is mainly a classification of agencies of disease rather than of diseases themselves. Chapter V very properly emphasizes the desirability of appraising the relative importance of forest ("tree") diseases in harmony with the aims and practices of sound forestry. The citation of "the white pine blister rust and the chestnut blight" as "the most important of all of our tree diseases" may be misleading in a way not intended. The latter, because of the thorough destruction it wrought, is now relatively unimportant in forestry. As for the former, one may well hesitate to give it first place on

the list if such wide-spread and often wholesale diseases are considered as those caused by such fungi as *Trametes Pini* and *Fomes igniarius*, involving as they do almost the entire range of our forests, softwood and hardwood. These and certain others render entire stands of vast area unsound, often to a degree of utter worthlessness. Chapter VI concisely sets forth the economic losses from forest diseases. The final chapter of Part I, Symptoms, devotes much space to an exposition of the etiology of decay in trees and in structural timber. The first part of this long chapter presents an analytical key to diseases of forest trees (11 pages) based on external symptoms. While duly recognizing the originality of this scheme, experience alone can demonstrate its usability. Hartig and others have accomplished essentially the same purpose by including at the end of their texts lists of diseases, with their obvious symptoms under alphabetically arranged host names. Such lists have the advantage of brevity and directness.

Part II is devoted to a detailed description of forest diseases; the subject matter is comprised in 3 chapters, Chapter VIII dealing with those caused by "non-organic or physiogenic agencies," Chapter IX with those caused by "organic agencies," and Chapter X with "control." Chapter IX, (pp. 142–439), by far the longest in the book, is arranged according to "the taxonomic order of the causal organism." As might be expected, the treatment of the many known diseases is uneven, partly by design and partly through necessity. There are great differences in the relative importance of forest diseases, and the vast majority have so far scarcely been studied at all. Turning over the pages, one is struck by the large numbers that have been seen, and even more so by the fewness of those that have been investigated. In dealing with subject-matter of this kind an author is confronted with the choice of 2 methods, namely, commenting briefly on all, as Hubert, for the main part, has done, or limiting descriptions to a selected, representative few, treating each at considerable length and relegating the rest to bare enumerations at the ends of appropriate sections, as Professor Heald has done in his Manual of Plant Pathology. Possibly Professor Hubert's choice is wise, considering the matter at his disposal. Under the various topics discussed the author describes the symptoms, the etiology, and, wherever possible, known or likely methods of control. The text is abundantly interspersed with literature lists. It may be suggested that Chapters VIII and IX are not quite accurately titled, a suggestion that applies to several other chapters in the book.

Part III presents the subject of "Wood Pathology." Here the author is at his best. Part III falls into 5 chapters, namely, The Properties of Wood Affected by Decay, The Relative Resistance of Wood to Decay, deterioration caused by "saprophytic agencies" (dry rot, decay of timbers, etc.), deterioration caused by "semiparasitic agencies" (sap stains of

wood), and "control." Eventually, this subject will almost certainly demand a separate volume to itself. Just now, however, it is a welcome inclusion here, because there is yet no consistent treatment of it elsewhere.

All in all, An Outline of Forest Pathology is a creditable volume. It will no doubt be extensively used by students of forestry and will find a place on the reference bookshelf of others who are interested in conservation, tree surgery, wood preservation, pulp and paper manufacturing, etc., as hoped by the author. As a contribution to forest pathology, proper, in America, it shows that enough already has been accomplished to place the subject on an established footing. We now know that a multitude of diseases of one kind or another threaten all forest age classes; even certain butt or heart rots are known to infest comparatively young stands; the majority of these diseases await investigation, especially from the standpoint of control. It is reasonable to anticipate that concentrated research on them, one after the other, will reveal both their etiology and practical methods of control. Obviously, a wider acquaintance with diseases of forest trees, forests as units, and forest soils is bound to be essential to the highest type of forestry practice as applied to reforesting, conservation, and utilization.—J. H. FAULL, Arnold Arboretum, Harvard University, Boston, Mass.